BALANCE AND GAIT
IN NEURODEGENERATIVE DISEASE

what startle tells us about motor control

Jorik Nonnekes

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Balance and gait in neurodegenerative disease: what startle tells us about motor control

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General introduction and outline of the thesis

Introduction

Motor control is the set of processes by which movements in human beings are produced and regulated. Most people are able to make movements without any problems. In contrast, motor control is hampered in patients with a wide variety of neurological diseases. Gait and balance impairments are among the most frequent and debilitating symptoms in these patients. They may result in falls and fall-related injuries that range from relatively innocent bruises to fractures or head trauma. Another consequence of gait and balance impairments is a limitation in mobility, which is often further reduced by fear of falling.[178] Reduced mobility leads to a loss of independence, deterioration of cardiovascular fitness, and promotes development of osteoporosis. Osteoporosis in turn increases the risk of future fractures following a fall. Both fractures and reduced cardiovascular fitness are associated with an increased morbidity and reduced quality of life,[352] and even increased mortality.

The mechanisms underlying deficits in motor control, and in particular the mechanisms underlying gait and balance impairments, are not well understood. Moreover, symptomatic treatment is limited. To develop improved treatment strategies, more insight is needed into the underlying mechanisms. To this aim, in this thesis, motor control is studied in three different groups: healthy subjects, people with hereditary spastic paraplegia (HSP), and people with Parkinson's disease (PD). Studies in healthy subjects were essential to investigate unaffected motor control. Studies in HSP and PD allowed me to study motor control in two different neurodegenerative disorders that both develop slowly, but that affect different neural structures. In HSP, the corticospinal (pyramidal) tract is affected bilaterally.[149,228] HSP is therefore termed a 'pyramidal disease'. In contrast, PD is a typical example of an 'extrapyramidal disease', which means that brain structures outside the pyramidal tract are affected. PD typically starts unilaterally, but soon develops into a bilateral condition.[172] Hence, by performing studies in PD and HSP, it was possible to investigate gait and balance deficits in both pyramidal and extrapyramidal diseases. In particular, I was interested in the role of brainstem structures in deficits in motor control. Based on previous studies in humans and animals,[275,387] I hypothesized that dysfunction of brainstem structures might contribute to gait and balance problems in PD, whereas they could serve a compensatory role in the case of HSP.

An important method that was used to study motor control was the startle reflex and the StartReact paradigm.[359] In this introduction, the StartReact paradigm will therefore be explained first. Then, dynamic posturography will be introduced as a method to study balance deficits. Thereafter, the clinical characteristics of HSP and PD will be introduced, with an emphasis on gait and balance impairments. Finally, the outline of this thesis is described based on the main research questions and hypotheses.

The startle reflex and StartReact paradigm

The startle reflex is an involuntary motor reaction to unexpected sensory input and consists of a generalized flexion response.[359] It is the fastest generalized motor reaction in humans and results from the activation of reticulospinal motor tracts in the pontomedullary reticular formation (pmRF) located in the brainstem.[194,386] Startle reflex activity is most prominent in the sternocleidomastoid muscle. Subsequently, the descending volley may activate more distal muscles in the trunk and upper and lower extremities.[41] The occurrence of reflex activity in distal leg muscles varies according to posture and is larger in a standing than in a sitting position.[41,89]

Startling stimuli can accelerate voluntary reaction times when delivered simultaneously with an imperative cue in a reaction time task, a phenomenon known as 'Start-React'.[356,357] The first StartReact experiments involved the acceleration of voluntary arm movements.[54,55,355,356] Experiments have shown that more complex movements, such as rising from sit-to-stance, gait initiation, stepping and obstacle avoidance can be accelerated by a startling stimulus as well.[209,293,300] The underlying mechanism of the StartReact phenomenon is not completely clear, but the predominant hypothesis is that movements are 'stored' in a pre-prepared state in the brainstem reticular formation where they can be released by the startle.[55,356] Hence, the StartReact paradigm enables us to study the functioning of the brainstem reticular formation and its role in motor control.

Although gait initiation and obstacle avoidance can be accelerated by a startling stimulus, it is not known whether postural responses to balance perturbations can be accelerated as well. In addition to StartReact responses, automatic postural responses to balance perturbations are likely to be evoked from the reticular formation.[337] As such, I hypothesized that postural responses could also be accelerated by a startling stimulus.

Dynamic posturography

Postural responses can be studied using dynamic posturography. Dynamic posturography is an umbrella term for a variety of techniques that employ physical perturbations of stance.[28] This is usually achieved by using motorized platforms of which the support surface (upon which the subject is standing) can be moved suddenly by powerful torque motors that assure standardised delivery of perturbations. In this thesis, we evoked balance perturbations by translating a unique, self-developed moveable platform (the Radboud Falls Simulator) in the forward or backward direction (Figure 1).

In posturography experiments, balance reactions can be measured quantitatively and in an objective manner. Postural reactions can be examined by analyzing forces

Figure 1 The Radboud Falls Simulator.

obtained from force platforms and by recording muscle activation patterns using surface-based electromyography. In addition, balance control can be quantified by analyzing the kinematics of the body parts. This can be accomplished by attaching markers to the participant's body that are tracked by a 3D camera system.

Hereditary spastic paraplegia

HSP is a diverse group of inherited disorders that are clinically characterized clinically by progressive lower-extremity spasticity and weakness.[150,311] The common pathological feature of these conditions is retrograde axonal degeneration of the corticospinal tracts, posterior spinal columns, and to a lesser extent the spinocerebellar fibers.[149,228] HSP can be divided into pure (uncomplicated) and complicated forms, depending on the presence of other neurological symptoms in addition to spastic paraparesis. Other neurological symptoms can be ataxia, severe amyotrophy, optic atrophy, mental retardation, extrapyramidal signs, dementia, deafness, peripheral neuropathy, and epilepsy.[311] HSP can be inherited as an autosomal dominant, recessive, or X-linked recessive trait.[311] Most cases of pure HSP are autosomal dominant, whereas complicated forms are rare and tend to be autosomal recessive. The axonal degeneration in HSP is

likely caused by disturbed membrane trafficking processes leading to abnormal axonal development, growth and maintenance, and eventually to axonal degeneration.[21] The main treatments of patients with HSP consist of administration of spasmolytic drugs (either orally, intrathecally, or delivered by injections) besides physiotherapy and exercise therapy to maintain muscle length, joint mobility and gait and balance capacity**.** These treatments are, however, symptomatic and do not cure or slowdown the disease itself. The disease onset is from early childhood up to 70 years of age. The first presenting symptoms are subtle with development of leg stiffness and minor gait impairments. As the disease progresses, balance impairments become very common. It has been shown that both spasticity and muscle weakness contribute to postural instability in HSP, but these cannot explain the balance impairments entirely.[86]

Parkinson's disease

PD is a neurodegenerative disorder characterized by both motor and non-motor symptoms. It is often recognized by its motor symptoms: rest tremor, bradykinesia and rigidity.[172] As the disease progresses, postural instability and gait disturbances are also frequently seen. Importantly, PD patients can also experience a wide range of non-motor symptoms such a reduced ability to smell, sleep disorders, cognitive deficits and mood disorders. These motor and non-motor symptoms in PD can be attributed mainly to dopaminergic depletion in the substantia nigra and the nigrostriatal pathway to the striatum (caudate nucleus and putamen). However, other central neurotransmitter systems such as noradrenergic, cholinergic and serotonergic systems may also become affected, and such extranigral lesions become more prominent with disease progression.[306]

PD is mostly seen above the age of 60 years, but it can also affect younger people. The main treatments include pharmacotherapy (mainly levodopa and dopamine agonists), deep brain stimulation (for a selected subgroup of patients), and multidisciplinary support (including, among others, Parkinson nurses, allied health professionals and social workers). As with HSP, these are symptomatic treatments that do not cure or slow down the disease itself.

Freezing of gait

Freezing of gait (FOG) is frequently seen in patients with PD. FOG is an episodic gait disorder where patients experience sudden and often unexpected episodes during which their feet are subjectively 'being glued to the floor' while their trunk tends to move forward.[215] In daily life, FOG usually occurs when starting to walk, when turning, or when walking in tight quarters. Turning around on the same spot appears to be the strongest provoking factor for FOG.[313] Most patients have dopamine-responsive FOG, which means that they have more frequent and severe episodes when the effect of medication has worn off. A small proportion of patients has dopamine-

induced FOG (caused by the administration of dopaminergic medication), or dopamine-resistant FOG (related to presence of non-dopmainergic brain lesions). The precise mechanisms underlying FOG are unknown. Most likely, FOG is not the result of one specific lesion in the brain, but rather results from dysfunction in the complex neural circuitry that is involved in gait regulation.[275] Clinicians invariably perceive treatment of FOG as a very challenging task, and this challenge is compounded by the lack of clear treatment protocols.

Postural instability in PD

Postural instability is common in PD and, together with FOG, is a main risk factor for falls.[224,288] PD patients are predominantly unstable in the backward direction, [62,167] and seem to have fundamental problems in the scaling of their postural responses.[2,3,62,100,175,191,192,336] It has been suggested that abnormal central proprioceptive-motor integration (rather than deficiencies in the afferent proprioceptive information itself) plays a role in the abnormal scaling of the balance responses.[31,175,189] However, it has not been unravelled which neural lesions primarily underlie postural instability in PD.

Outline of this thesis

The aim of this thesis was to study the mechanisms underlying deficits in motor control in both pyramidal and extrapyramidal neurodegenerative diseases, with an emphasis on gait and balance impairments. First, motor control in healthy subjects was studied, as described in ***part 1*** of this thesis. In **chapter 2**, I investigated why the occurrence of startle reflex activity in distal leg muscles varies according to posture. It was hypothesized that the amount of loading of a leg influences the occurrence of startle responses in the leg. In **chapter 3**, I studied whether the StartReact effect is also applicable to postural responses. For reasons described in chapter 3, I expected that the effects of the startle would be more prominent in responses to backward than to forward perturbations. In **chapter 4**, I explored whether transcranial direct current stimulation (tDCS, see box. 1) is able to facilitate subcortical structures, in particular the reticular formation. As tDCS is able to facilitate the reticular formation in cats[36], it was hypothesized that this might be possible in humans as well.

Box 1 Transcranial direct current stimulation

Transcranial direct current stimulation (tDCS) is a technique that allows stimulation of the brain from outside the head. Weak electrical direct currents (< 5 mA) are applied to the head using two skin electrodes. These weak currents can slightly increase or decrease the neural excitability in the brain areas underlying the electrodes, depending on the polarity of the applied current.[19,78,291] If tDCS is continuously applied for a longer duration (for example 15 minutes) these effects can last up to one hour after the stimulation. tDCS has been intensively studied in the last decade and has shown to be safe. It is an important technique for neuroscientists to investigate the function of cortical structures.

Part 2 of this thesis describes two studies that were performed in patients with HSP. In **chapter 5**, patients with HSP were used as a model to study different hypothesis explaining the StartReact effect. I expected that our results would provide evidence for the hypothesis that the StartReact effect is due to the startle releasing a subcortically stored motor program, which is being conveyed by the reticulospinal tract. In **chapter 6** I examined balance responses in patients with HSP. I investigated the hypothesis that delayed postural responses contribute to balance impairments in these patients. In addition, I aimed to distinguish between a possible delay of signals in the afferent (posterior spinal columns) or efferent (reticulospinal) tracts, using balance perturbations both with and without a concurrent startle.

Studies on gait and balance problems in PD are described in ***part 3*** of this thesis. **Chapter 7** includes a narrative review on postural instability in PD and describes how dynamic posturography can help to unravel the underlying mechanisms. In **chapter 8**, FOG is studied. I explored the hypothesis that dysfunction of upper brainstem structures contributes to FOG. The function of these structures was examined by applying the StartReact paradigm to gait initiation. In **chapter 9**, the StartReact paradigm was used to explore whether dysfunction of brainstem structures contributes to the balance impairments in PD as well.

In **Chapter 10**, I review medical and non-medical treatment strategies for freezing of gait and present a practical algorithm for the management of this disorder.

Part 4 of this thesis provides an overview. In **chapter 11**, I provide a comprehensive review on startle reflexes and StartReact, and their interaction with posture and gait. In **chapter 12** the results of this thesis are summarized and placed in a broader perspective. A Dutch summary is given in **chapter 13.**

PART 1

Healthy controls

2

Loading enhances the occurrence of startle responses in leg muscles

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Loading enhances the occurrence of startle responses in leg muscles.
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Abstract

The startle reflex is an involuntary reaction to sudden sensory input and consists of a generalized flexion response. Startle responses in distal leg muscles occur more frequently during standing compared to sitting. We hypothesized that sensory input from load receptors modulates the occurrence of startle responses in leg muscles.
We administered sudden startling auditory stimuli (SAS) to 11 healthy subjects while (1) sitting relaxed, (2) standing relaxed, (3) standing while bearing 60% of their weight on the right leg, (4) standing while bearing 60% of their weight on the left leg, and (5) standing with 30% body weight support ('bilateral unloaded'). The requested weight distribution for each condition was verified using force plates. Electromyography (EMG) data were collected from both tibialis anterior (TA) and the left sternocleido-mastoid muscles.
In the TA, startle responses occurred much more frequently during normal standing (26% of trials) compared to both sitting (6% of trials, $p<0.01$) and bilateral unloading (3% of trials, $p<0.01$). In the asymmetrical stance conditions, startle responses in the TA were more common in the loaded leg (21% of trials) compared to the unloaded leg (10% of trials, $p<0.05$).
The occurrence of startle responses in the leg muscles was strongly influenced by load. Hence, it is likely that information from load receptors influences startle response activity. We suggest that, in a stationary position, startling stimuli result in a descending volley from brainstem circuits, which is gated at the spinal level by afferent input from load receptors.

Introduction

The startle reflex is a generalized flexion response to sudden sensory input and is the fastest generalized motor reaction in humans and animals.[359] The startle reflex arises from the pontomedullary reticular formation[83,386] and the latencies of the muscle responses increase with the distance of the respective muscle from the caudal brainstem.[42] Interestingly, startle responses in distal leg muscles occur more frequently in a standing position compared to sitting relaxed.[41,89] Therefore, it has been suggested that the function of the startle responses in distal leg muscles lies in rapidly accomplishing a defensive stance with maximum postural stability.[41] However, it is unknown how the startle reflex is modified during various postures.

We hypothesized that the amount of loading of a leg contributes to the occurrence of startle responses in leg muscles. A large body of evidence exists that leg muscle activity can be modulated by various loading conditions.[98,106] For example, an increase in loading yielded larger amplitudes of postural responses.[94,170] Furthermore, the observation that startle responses during gait are larger during the stance compared to the swing phase may also be suggestive of load-induced modulation.[260,314] We aimed to seek further evidence for this hypothesis by investigating startle reflexes during various loading conditions of the legs.

Experimental procedures

Participants

Participants in this study were 11 healthy adults (seven women, four men; mean 24 years, range 20-28 years). None of them suffered from any hearing, neurological or motor disorder that could interfere with their performance during the experiments. All subjects gave written informed consent prior to the experiment. The experiments conformed with the standards of the Declaration of Helsinki and with local ethical guidelines.

Experimental setup and protocol

Startling auditory stimuli (SAS) were given in five different body positions ('conditions'). In each condition four SAS were delivered. Subjects were either (1) sitting quietly (in a chair with backrest), (2) standing quietly (with equal weight distribution), (3) standing while bearing 60% of their weight on the right leg (40% on the left leg), (4) standing while bearing 60% of their weight on the left leg (40% on the right leg) or, (5) standing symmetrically with 30% body weight support ('bilateral unloaded'). Bilateral unloading was achieved by suspending the participants from a parachute harness connected

to an overhead crane. Conditions 3 and 4 are referred to as 'unilateral loaded'. We chose for the subtle 60-40% weight distribution in these unilateral loaded conditions, to ensure that both legs were still involved in the regulation of posture.[8] During conditions 2-5, weight distribution was monitored using two force plates and feedback was given to the participants. Acceptable deviations were within ± 5%.

The 20 SAS were given divided over three sessions (6 or 7 stimuli per session) on separate days, to prevent habituation of the startle reflex. Each session comprised a maximum of two trials of the same conditions and the order of the conditions was varied between sessions. Within one session, the period between two subsequent SAS was approximately 5 minutes. The SAS were given through binaural earphones and consisted of 50 ms of white noise with an intensity of 117 dB (SPL), generated by a custom-made noise generator. Acoustic stimuli are commonly used to evoke startle response.[41,54,356]

Data collection. Electromyography (EMG) data were collected from bilateral tibialis anterior (TA) and left sternocleidomastoid (SCM) muscles. Self-adhesive Ag-AgCl electrodes (Tyco Arbo ECG) were placed approximately 2 cm apart and longitudinally on the belly of each muscle, according to Seniam guidelines.[156] EMG signals were sampled at 2000 Hz and band-pass filtered at 10 and 500 Hz.

Data analysis. Two observers, who were blinded for the conditions, independently identified startle-induced responses from the EMG signals and, if present, they determined the onset latencies. A response had to occur within 100 ms after SAS for the SCM[42,349] and within 180 ms for the TA.[41] For each muscle, the rate of occurrence of startle-induced activity was determined as the percentage of trials in which a response could be identified. The rates of occurrence and onset latencies were averaged for both raters; the rate of occurrence did not differ between the two raters, the maximum deviation of latencies was 4 ms. In addition, for each condition the mean background EMG activity in the TA was determined over a period of 500 ms before the SAS.

Statistical analysis. The occurrences of startle responses were analyzed using Pearson's Chi-square tests. The onset of SCM responses and background EMG activity in TA were compared between the various conditions using a repeated measures analysis of variance (ANOVA) with 'Condition' as a within-subjects factor. The alpha level was set at 0.05.

Results

Occurrence of startle responses

The SAS resulted in startle responses in the SCM in 77% (bilateral unloaded) to 93% (quiet sitting and standing) of the trials (Figure 2a). The occurrence of startle responses in the SCM did not differ between the quiet sitting, quiet standing and unilateral loaded conditions ($p > 0.18$). However, the occurrence of the startle responses in the SCM was less frequent in the bilateral unloaded condition compared to quiet standing ($p=0.034$)

There was an absence of background activity in the TA in all conditions (see Figure 1); the mean activity varied between 0.0045 mV and 0.0059 mV (no significant differences between conditions; $F_{4,7}=2.107$; $p=0.183$). TA responses occurred much more frequently during quiet standing (26%) compared to sitting (6%) ($p<0.010$) and bilateral unloading (3%) ($p<0.004$; Figure 2b). In the symmetrical standing condition, the rates of occurrence of TA responses did not differ between the left (27%) and right leg (25%; $p=0.81$). In the unilateral loaded conditions, TA responses were more prevalent in the loaded (21%) compared to the unloaded leg (10%; $p = 0.033$; Figure 2b).

Figure 1 EMG traces of a representative subject during the sitting, stance and bilateral unloaded conditions, for both the sternocleidomastoid (SCM, left panels) and tibialis anterior muscle (TA, right panels). For these trials the latencies were determined as follows: sitting SCM=50 ms; stance SCM=53 ms; bilateral unloaded SCM=54 ms; stance TA=100 ms.

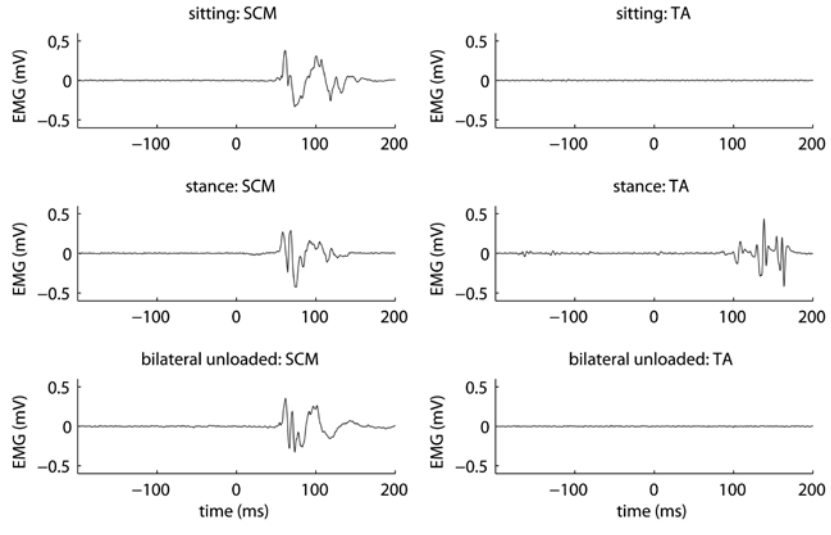
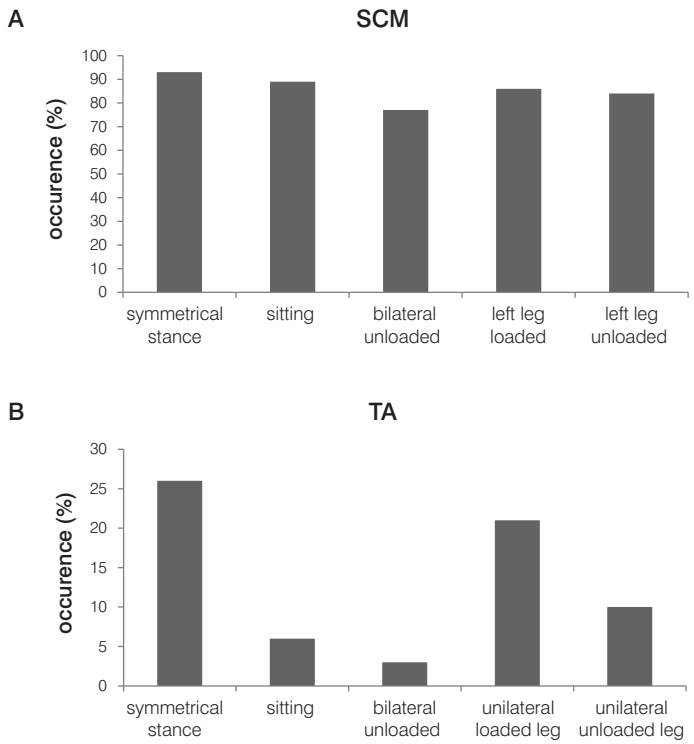


Figure 2 Occurrence of startle responses (**A**) in the sternocleidomastoid muscle (SCM) and (**B**) in the tibialis anterior muscle (TA).



Latencies of startle responses

In Table 1, the mean onset latencies of muscle activity are listed for the various conditions. There were no significant differences in SCM latencies between the various conditions ($F_{4,6}$=0.360, p=0.828). Latencies in TA tended to be delayed in the quiet sitting and bilateral unloaded conditions compared to the quiet standing condition, but no statistics were performed due to the low occurrence of responses in the sitting and bilateral unloaded conditions. TA latencies did not differ between the loaded and unloaded leg in the unilateral loading conditions.

Table 1 Mean onset latencies (ms) of startle responses in SCM and TA.

	Onset SCM	Onset TA
Stance	72 ± 8	101 ± 27
Sitting	60 ± 7	130 ± 24
Bilateral unloaded	62 ± 8	129 ± 19
Unilateral loaded	Left leg loaded 61 ± 7 Left leg unloaded 60 ± 10	Loaded leg 115 ± 31 Unloaded leg 112 ± 37

Discussion

The present study demonstrated that increased leg loading resulted in more frequent occurrence of startle responses in the tibialis anterior (TA) muscles. Interestingly, this was not only true for symmetrical standing compared to sitting and bilateral unloading, but also for the loaded versus unloaded leg when participants adopted an asymmetrical (60-40% weight distribution) standing position.

Previous research has also demonstrated that the occurrence of startle responses in leg muscles (tibialis anterior and soleus) is affected by posture. EMG activity in the lower leg muscles was found to be more prevalent while standing compared to sitting, whereas rates of occurrence of reflex EMG activity in sternocleidomastoid did not differ.[41,89] In our study, the occurrence of the startle responses in the SCM was less frequent in the bilateral unloaded condition compared to quiet standing. Due to the suspension of the participants a slight forward head posture may have occurred during the bilateral unloaded condition, resulting in fewer startle responses. Furthermore, the previous studies observed shorter latencies of reflex EMG activity while standing (70-95 ms) compared to sitting (120 ms).[41,89] Our results are in line with these observations.

The present study extends previous work by showing that more subtle changes in posture (i.e., weight-bearing asymmetry) may also have a profound effect on the occurrence of startle responses in the legs. This observation is reminiscent of previous research on phase-dependent modulation of startle reflex expression during gait.[260,314] These studies demonstrated that startle-induced responses in the leg muscles occurred bilaterally, but appeared more prominent in the stance leg compared to the swing leg. Differences in background EMG between gait phases were suggested to possibly underlie these results, but findings from other studies[41,89] and the present study argues against this explanation. Furthermore, Nieuwenhuijzen

and co-workers showed that background variations could not account for all features of the modulation of the responses and hypothesized that during walking, the phase-dependent state of the central pattern generator might modulate the expression of startle responses.[260] This hypothesis is also not likely to explain the differences in startle expression between the loaded and unloaded leg during asymmetrical stance in our experiment. Our findings rather point toward a critical role of afferent (loading) information from the legs underlying phase-dependent modulation of startle reflexes during gait. Likely, load-related efferent information is also used in the modulation of other reflex responses, as previous studies reported on load-related modulation of cutaneous reflexes.[14,248]

The present results raise the question of how the modulation of the startle reflex would be organized in the central nervous system. Startling stimuli elicit a descending volley from the pontomedullary reticular formation (pmRF)[83,200], which is conveyed by the reticulospinal tract and projects onto spinal interneurons. Both the pmRF and spinal interneurons receive afferent information from the legs.[103] Information on the amount of loading of a leg is provided by Ib afferents from Golgi tendon organs and cutaneous mechanoreceptors in the foot soles.[98,106,181] This afferent information may serve as the input for modulation of the startle reflex, but would this process take place in the brainstem or at the level of the interneurons in the spinal cord? Studies in cats suggest the latter, as descending symmetrical volleys from startle circuits were found to be gated at premotoneural level by spinal interneuronal networks.[102,316] Our observation that the occurrence of startle responses differs between both legs when the load is shifted toward one leg, without changing the responses in sternocleidomastoid, is in line with this idea.

The posture and loading-related effects are not exclusive for the modulation of startle reflex expression, but also pertain to other descending reticulospinal signals, for example those that contribute to postural adjustments.[96] In the latter study it was shown that loading yields larger postural responses after translational perturbations. Furthermore, in another experiment, Dietz and Colombo studied postural adjustments associated with pull and push arm movements in a reaction time task while floating and while standing or sitting out of water.[97] Reaction times were longer in a sitting compared to a standing position, which was related to postural adjustments required while standing. These postural adjustments did not occur under free-floating conditions, but the reaction times remained longer compared with the sitting condition. As in our experiment, it was suggested that gating of supraspinal signals to leg muscles takes place at the spinal level on the basis of afferent information signaling the amount of leg loading. It remains for further research to determine which peripheral receptors provide the afferent loading information.

The functional implications of the present findings remain speculative. The startle reflex can be described as a distributed set of automatic responses to an environmental disturbance[110] and, as such, can have different functions. Our results support the idea that one of the functions of the startle reflex lies in rapidly accomplishing a defensive stance with maximal stability.[41] To accomplish maximal postural stability, leg muscle activation is only useful when someone is actually standing. In the case of an asymmetrical weight distribution of the legs, fast activation of the muscles in the loaded leg is most effective as they yield the largest corrective torques.
A limitation of this study is that we recorded startle responses from tibialis anterior only. We chose to do so as the background activity in this muscle during both sitting and standing is small compared to other leg muscles, which allows for the detection of even very subtle startle responses. However, our results raise the question whether the load-dependent modulation of startle responses also pertains to other leg muscles.

In conclusion, the present study shows that loading increases the occurrence of startle responses in distal leg muscles. We suggest that startling stimuli result in a bilateral descending volley from brainstem startle circuits that are gated at the spinal level by afferent input from load receptors.

3

Are postural responses to backward and forward perturbations processed by different neural circuits?

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Abstract

Startle pathways may contribute to rapid accomplishment of postural stability. Here we investigate the possible influence of a startling auditory stimulus (SAS) on postural responses. We formulated four specific questions: (1) can a concurrent SAS shorten the onset of automatic postural responses?; and if so (2) is this effect different for forward versus backward perturbations?; (3) does this effect depend on prior knowledge of the perturbation direction?; and (4) is this effect different for low- and high-magnitude perturbations?

Balance was perturbed in 11 healthy participants by a movable platform that suddenly translated forward or backward. Each participant received 160 perturbations, 25% of which were combined with a SAS. We varied the direction and magnitude of the perturbations, as well as the prior knowledge of perturbation direction. Perturbation trials were interspersed with SAS-only trials.

The SAS accelerated and strengthened postural responses with clear functional benefits (better balance control), but this was only true for responses that protected against falling backwards (i.e. in tibialis anterior and rectus femoris). These muscles also demonstrated the most common SAS-triggered responses without perturbation. Increasing the perturbation magnitude accelerated postural responses, but again with a larger acceleration for backward perturbations.

We conclude that postural responses to backward and forward perturbations may be processed by different neural circuits, with influence of startle pathways on postural responses to backward perturbations. These findings give directions for future studies investigating whether deficits in startle pathways may explain the prominent backward instability seen in patients with Parkinson's disease and progressive supranuclear palsy.

Introduction

The startle reflex is an involuntary reaction to an unexpected sensory input and is the fastest generalized motor reaction of humans and animals.[359] Brainstem startle pathways may play a role in the rapid accomplishment of postural stability.[41,280,349] It is conceivable that the startle reflex and postural responses share common neural circuits, but this has never been investigated.

When a startling auditory stimulus (SAS) is used to elicit the startle reflex, neurons from the cochlear nucleus synapse on the pontomedullary reticular formation (pmRF).[194,386] Interestingly, the giant neurons of the pmRF are not modality specific[380] and may therefore be activated by other sensory stimuli as well. In cats, cells in the pmRF discharge in response to unexpected lowering of the support surface under a single limb, and their activity likely contributes to compensatory postural responses.[161,337] In humans, automated postural responses may also arise from the brainstem,[176] but the exact neural circuits remain to be identified.

The suggestion that the startle reflex and postural responses may share neural circuits is supported by the observation that patients with progressive supranuclear palsy (PSP) have postural instability as well as reduced or absent startle reflexes.[195] Postural instability is also common in patients with Parkinson's disease (PD), and it seems that patients with postural instability (and freezing of gait) do have absent startle reflexes as well,[349] whereas on the other hand a smaller habituation rate of startle reflexes has been reported in patients with mild PD.[261] Interestingly, both PSP-patients and PD-patients with postural instability are more unstable in the backward direction than in other directions.[25,62,100] A similar directional sensitivity has also been observed during 'first trial' balance perturbations in healthy subjects. 'First trial' perturbations evoke startle-like responses, but these were particularly evident for backward balance perturbations.[279] These observations suggest that particularly postural responses to backward balance perturbations may be linked to startle circuits.

Here we investigated the presumed interaction between startle and postural responses, using the StartReact paradigm. In this paradigm, a SAS is delivered at the same time as the imperative cue, resulting in an acceleration, and sometimes an augmentation, of the response.[198,355,356] This StartReact phenomenon likely operates via the SAS, which directly releases a prepared motor response.[54,356] We investigated whether a SAS would also accelerate and augment postural responses to translational balance perturbations. For reasons described above, we expected that the effects of the SAS would be more prominent in responses to backward than to forward perturbations.

We also compared the effects of a SAS between low and high-magnitude perturbations. Furthermore, we determined whether SAS-related effects would depend on advance knowledge of the direction of the perturbation. Finally, we investigated whether we could trigger postural responses by a SAS in the absence of a perturbation.

Experimental procedures

Participants

Eleven healthy adults (8 women, mean 26.4 years, range 22-32) participated. None of them suffered from hearing, neurological or motor disorders that could interfere with performance during the experiments. The mean weight of the participants was 74 kg (range 61-127 kg), average height was 1.77 m (1.66-1.93 m) and average BMI was 24 kg/m^2 (range 18-35 kg/m^2). The study was approved by the local medical ethics committee and was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent prior to the experiment.

Experimental setup and protocol

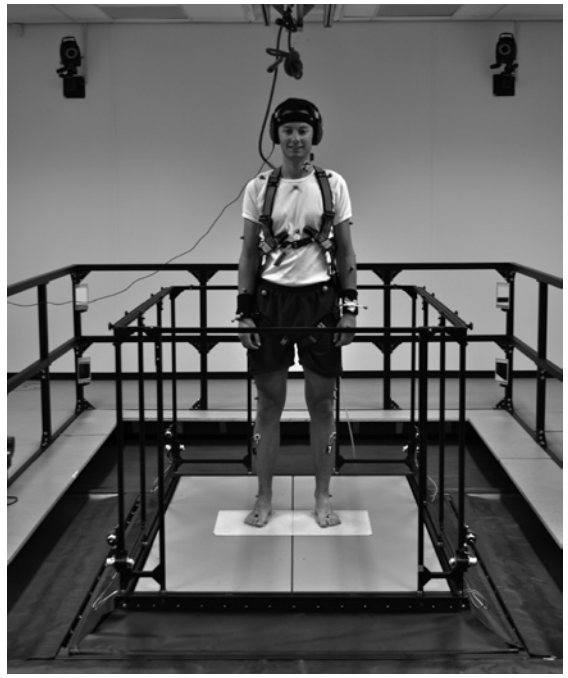
Participants stood on a moveable platform (138 by 191 cm, Baat Medical, Enschede, The Netherlands; Figure 1) with two embedded force plates (60 by 180 cm each, AMTI Custom 6 axis composite force platform, USA). The platform could suddenly and unexpectedly translate in the forward or backward direction. A forward translation of the platform resulted in a backward balance perturbation and a backward translation resulted in a forward balance perturbation; we will refer to the direction of the balance perturbation. Platform movements comprised an acceleration phase of 300 ms, followed by a constant velocity phase of 500 ms and a deceleration phase of 300 ms.

Protocol

Participants received a total of 160 perturbation trials, with variable intertrial intervals (3-6 s). In 25% of all balance perturbations, the perturbation was combined with a startling auditory stimulus (SAS) that was randomly administered through binaural earphones at the start of the platform movement. The SAS consisted of 50 ms of white noise with an intensity of 116 dB and was generated using a custom-made noise generator.

Furthermore, we varied the perturbation direction (forward and backward), advance knowledge of the perturbation direction (known and unknown), as well as the perturbation magnitude (high and low acceleration). The low platform acceleration level was set at 0.5 m/s^2, as pilot experiments in 25 healthy young participants showed that they could all overcome this perturbation magnitude with a feet-in-place

Figure 1 Photograph of the balance perturbation platform. Force plates (visible as the two light-grey rectangles) were embedded in the platform.



response, whereas the high-magnitude perturbations of 1.75 m/s^2 required taking a step to prevent falling.

For the trials in which the perturbation direction was known in advance, there were four blocks of 20 trials (forward-low, forward-high, backward-low, and backward-high). The unknown direction perturbations were presented in two blocks of 40 trials (high and low), each block with 20 backward and 20 forward trials in random order. The blocks of trials were also presented in random order, and resting periods were provided between the different blocks. Additionally, three 'SAS-only' trials, in which a SAS was delivered without a platform movement, were randomly interspersed among each 20 perturbations. Consecutive SAS trials (with or without platform movement) were at least 20 seconds apart.

Participants were instructed to respond to the balance perturbation as they would do in daily life. Prior to the experiment, eight practice trials were administered to familiarize to the movements of the balance platform. Participants wore a safety harness and handrails were present to lend support in case of an actual fall.

Data collection

We recorded surface electromyography (EMG), kinematic and kinetic responses. In addition we recorded the platform movement and signal of the SAS.

EMG. Muscle activity was recorded bilaterally from the rectus femoris (RF), biceps femoris, tibialis anterior (TA) and the gastrocnemius medialis muscles, and unilaterally from the (left) sternocleidomastoid muscle (ZeroWire by Aurion, Italy). Self-adhesive Ag-AgCl electrodes (Tyco Arbo ECG) were placed approximately 2 cm apart and longitudinally on the belly of each muscle, according to the Seniam guidelines.[156] EMG signals were sampled at 1000 Hz, full-wave rectified and low-pass filtered at 20 Hz (zero-lag, second-order Butterworth filter).

Kinematics. Reflective markers were placed at anatomical landmarks according to the full-body Plug-in-Gait model.[84] Marker positions were recorded by an 8-camera 3D motion analysis system (Vicon Motion Systems, United Kingdom) at a sample rate of 100 Hz and low-pass filtered at 10 Hz (zero-lag, second-order Butterworth filter). We also placed one marker on the platform. Marker data were synchronously sampled with the EMG signals.

Kinetics. Ground reaction forces under both feet were recorded at a sample rate of 1000 Hz by two force plates (AMTI Custom 6 axis composite force platform, USA), which were embedded in the moveable platform. The force signals were low-pass filtered at 10 Hz (zero-lag, second-order Butterworth filter).

Data analysis

EMG. For each participant, each condition, and each muscle, we calculated ensemble average EMG traces, separately for trials with and without a SAS. Muscle onset latencies during trials with and without a SAS were determined using a semi-automatic computer algorithm that selected the instant at which the EMG activity first exceeded a threshold of 2 standard deviations (SDs) above the mean background activity, as calculated over a 100 ms period just prior to perturbation onset.[62,293] After being determined by the computer algorithm, onset latencies were visually checked and corrected as needed. We calculated the average maximum EMG amplitude over a period of 100 ms following the onset of muscle activity.[293] The test-retest reliability of the onset latencies and EMG amplitude was evaluated in a pilot experiment (Crohnbach's alpha 0.973 and 0.909, respectively).

For each condition, we also determined the percentage of 'SAS-only' trials in which activity rose, for more than 25 ms, above the mean background activity plus 2 SD (calculated over 100 ms prior to the SAS). We searched for tibialis anterior and

gastrocnemius medialis muscle latencies between 100 ms and 160 ms after SAS and for rectus femoris between 100 ms and 190 ms after SAS, as this was the time interval in which latencies occurred during perturbation trials. Note that the EMG data of one participant during expected backward perturbations with a low-magnitude could not be used for analysis due to technical malfunctioning.

Both in SAS-only trials as in perturbation trials with a SAS, we assessed the occurrence startle reflexes in the sternocleidomastoid muscle, as defined by the presence of a short latency response in the sternocleidomastoid muscle within 100 ms after the SAS.[42,349] The response had to sustain, for at least 20 ms, over a threshold of 2 SD above mean background activity, as calculated over a 500 ms period just prior to the SAS.

Kinematics. Due to frequent occlusions of the thigh markers by the rails mounted around the platform, we could not consistently calculate the centre of mass from the Vicon software. Therefore, we used the marker at the 7th cervical vertebra (C7) as a measure of the backward trunk displacement in the low-magnitude perturbation trials (i.e. feet-in-place responses). We subtracted the movement of the platform marker from the displacement of the C7 marker. The displacement of the trunk was then defined as the maximum displacement of the C7 marker.

For the high-magnitude perturbations, step onset was determined as the time between start of the platform movement and the time at which the heel marker moved backward (velocity $>$ 0.1 m/s). For each subject, the preferred stepping leg was determined as the leg that was most frequently used to take a step.

Kinetics. For the high-magnitude perturbations, we determined for each step whether an anticipatory postural adjustment (APA) occurred prior to step onset. A weight shift was considered an APA if it met two criteria. First, the difference between the vertical loading underneath the stance and stepping leg had to exceed a threshold of 2 SD above the mean difference, as calculated over a 100 ms period prior to perturbation onset. Second, an increase in force under the stepping leg had to exceed 5% of the total body weight. As such, normal changes due to weight shifts were not classified as an APA. If an APA occurred, we determined the maximum increase in vertical force under the stepping leg, normalized for body weight.

Statistical analysis

We compared the onset latencies of the prime movers of the backward and forward postural responses using a repeated measures ANOVA for the within-subject factors Startle (SAS versus no SAS), Expectation (perturbation direction known - unknown),

Leg (stance – stepping), Magnitude (low – high) and Direction (backward (tibialis anterior) – forward (gastrocnemius)). For the low-magnitude perturbations, the legs were classified as 'stance' or 'stepping' in accordance with the preferred stance and stepping leg during the high-magnitude perturbations.
For the low-magnitude perturbations, we investigated trunk displacement using a within-group ANOVA model for Startle (SAS versus no SAS), Expectation (perturbation direction known – unknown) and Direction (backward – forward).

Subsequent repeated measures ANOVAs and paired sampled t-tests were conducted to further explore the effects of the SAS on onset latencies, EMG response amplitudes and kine(ma)tic responses during backward perturbations. In these analyses we also used the within-subject factor Muscle (tibialis anterior – rectus femoris) to identify potential differential effects of the SAS on the various muscles involved in the postural response.

Rates of occurrence of muscle responses during 'SAS-only' trials were analyzed using a repeated measures ANOVA with within-subject factors Condition (expected backward – expected forward - unexpected direction), Muscle (TA – RF – GM) and Magnitude (low – high).

Results

Differential effects of SAS on responses to forward and backward perturbations

SAS shortened the onset-latencies of the prime mover following backward perturbations (tibialis anterior), but did not accelerate the onset of the prime mover following forward perturbations (gastrocnemius) (Figure 2). The SAS resulted in a significant 11 ms shortening of onset latencies in tibialis anterior following backward perturbations, but did not accelerate the gastrocnemius onset following forward perturbations (*startle x direction,* $F_{1,9} = 13.966$; $p = 0.006$; post-hoc *t* tests, $p(TA) = 0.008$, $p(GM) = 0.902$; Figure 3, Table 1). This effect was independent of prior knowledge of perturbation direction (*startle x direction x expectation*, $F_{1,9} = 0.202$; $p = 0.655$).
Onset latencies were also shortened by an increase in perturbation magnitude, and this reduction was significantly greater following backward perturbations (13 ms) than following forward perturbations (4 ms; *magnitude x direction;* $F_{1,9} = 18.829$; $p = 0.002$; post-hoc t tests, $p(TA) = p<0.001$, $p(GM)=0.039$, Table 1). This effect of perturbation magnitude was comparable for SAS and non-SAS trials (*startle x magnitude x direction*; $F_{1,9} = 1.296$, $p =0.288$).

Figure 2 Representative traces of EMG-activity recorded in tibialis anterior, rectus femoris, gastrocnemius, biceps femoris and sternocleidomastoid muscles of a single subject during low-magnitude perturbations in expected backward and forward direction. Black = perturbations with SAS. Grey = perturbations without SAS. Note that the y-axis of the tibalis anterior muscle has a different scaling for the forward and backward perturbations. None of the responses in the sternocleidmastoid muscle were classified as a startle reflex.

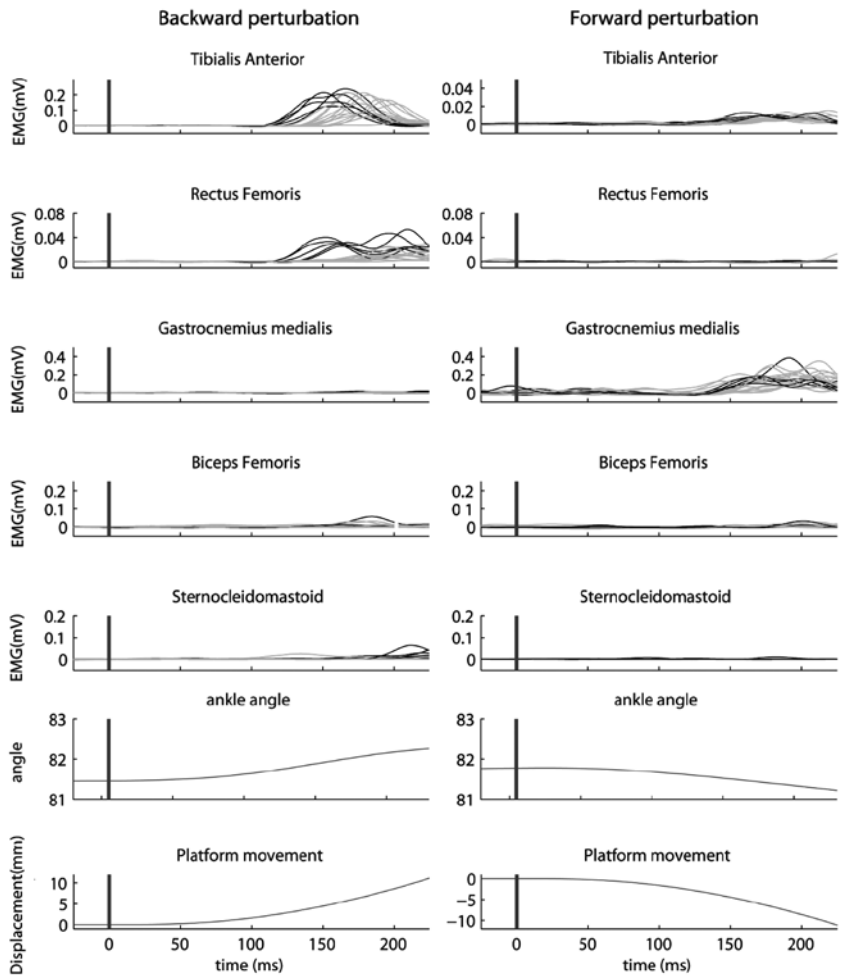


Figure 3 Mean onset latencies (SE) of the prime movers during backward (tibialis anterior, TA) and forward perturbations (gastrocnemius, GM). Dotted lines = low magnitude perturbations. Solid lines = high magnitude perturbations. * indicates significant differences.

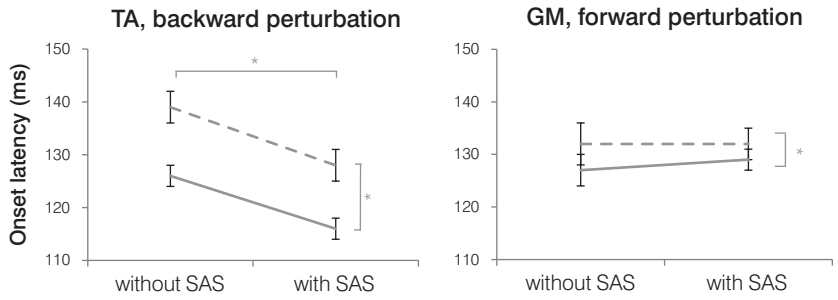


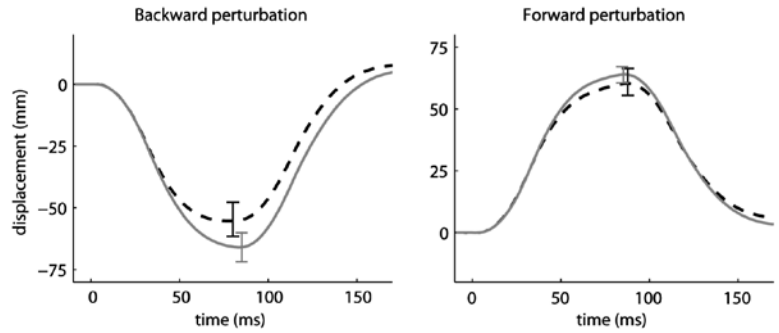
Table 1 Differences between forward and backward perturbations.

	SAS*	Magnitude+	Expectation
Backward perturbation			
Tibialis anterior onset	p=0.008	p<0.001‡	n.s.
Rectus femoris onset	p<0.001	p<0.001	n.s.
Biceps femoris onset	p=0.012	n.a.	n.s.
Step onset	n.s.	n.a.	n.s
Trunk displacement	p=0.002	n.a.	n.s.
Forward perturbation			
Gastrocnemius onset	n.s	p=0.039‡	n.s.
Tibialis anterior onset	p=0.001	p=0.001	n.s.
Trunk displacement	n.s.	n.a.	n.s.

n.s. = not significant; n.a.=not applicable
* significant differences indicate faster responses or smaller excursions with than without SAS.
+ significant differences indicate faster responses of high compared to low magnitude perturbations.
‡ larger magnitude effect on tibialis anterior onsets than on gastrocnemius onsets (direction x magnitude; p =0.002).

During perturbations with low magnitude, the trunk displacement (as recorded from a marker at the 7th cervical vertebra) did not differ between backward and forward perturbations (*direction*; $F_{1,9} = 0.008$; $p = 0.930$; Figure 4). The SAS reduced the trunk displacement during backward perturbations with 15% (58 ± 21 mm versus 68 ± 19 mm), but did not significantly reduce the trunk displacement during forward perturbations (*startle x direction,* $F_{1,9} = 5.993$; $p = 0.037$, post-hoc *t* tests, p(backward)=0.002, p(forward) = 0.128; Table 1). This effect was independent of prior knowledge of perturbation direction (*startle x direction x expectation*, $F_{1,9} = 0.959$; $p = 0.353$).

Figure 4 Group mean trunk displacement (as recorded from a marker at the 7th cervical vertebra) during backward and forward perturbations with low magnitude. Dotted black = perturbations with SAS. Grey = perturbations without SAS. Standard errors are shown at the point of maximum displacement.



The response to 'SAS-only' trials (without a perturbation)

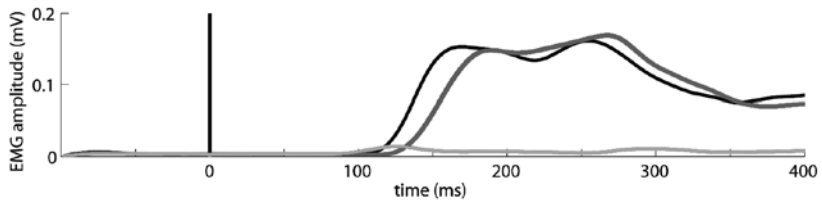
During 'SAS-only' trials, the occurrence of responses was highest in tibialis anterior (25%), followed by rectus femoris (15%) and gastrocnemius (10%; *muscle;* $F_{1,9} = 15.140$; $p = 0.004$; Table 2). In tibialis anterior and rectus femoris, startle responses during 'SAS-only' trials occurred more often in high than in low-magnitude perturbation blocks, particularly in those conditions that involved or could involve backward balance perturbations (*magnitude x muscle x condition,* $F_{4,6} = 6.592$; $p = 0.022$). We observed no clear pattern of habituation or sequence effects over the trials. The amplitude of the responses in tibialis anterior and rectus femoris were small compared to the amplitude recorded during the postural responses, both with and without SAS

Table 2 Percentage of 'SAS-only' trials with muscle activity.

		Backward perturbation direction known	Forward perturbation direction known	Perturbation direction unknown (backward and forward)
TA	Low	24%	18%	23%
	High	31%	18%	37%
RF	Low	14%	8%	13%
	High	20%	14%	18%
GM	Low	7%	11%	9%
	High	8%	14%	8%

(Figure 5). The amplitude was not only small when looking at the ensemble average traces, but also when looking at the individual trials.
The average onset of tibialis anterior in 'SAS-only' trials during low-magnitude conditions was 130 ± 24 ms versus 109 ± 25 ms during high-magnitude conditions. Due to the low rates of occurrence no statistics were performed.

Figure 5 Group mean EMG amplitude of tibialis anterior during the condition with high-magnitude perturbations in expected backward direction. Black = perturbations with SAS. Dark grey = perturbations without SAS. Light grey = 'SAS-only' trials.



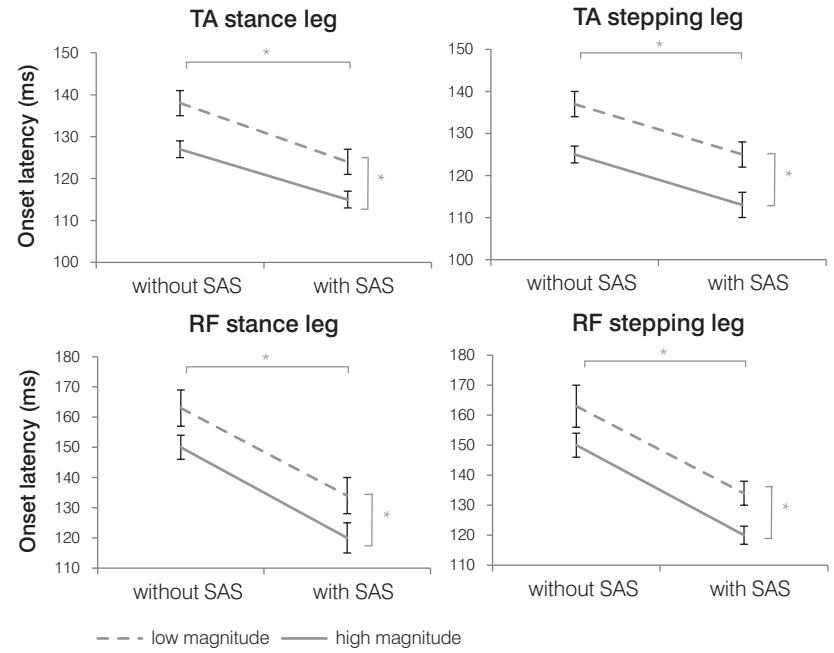
The effects of a SAS in expected low-magnitude backward perturbations

Backward trials not only activated tibialis anterior (onset latency 138 ± 10ms), but also consistently rectus femoris (at 160 ± 21 ms after perturbation onset). Accelerated response onsets were observed in both tibialis anterior and rectus femoris when a SAS was delivered simultaneously with the platform translation. The SAS did not result in a generalized muscle response as no response was seen in the gastrocnemius and biceps femoris (Figure 2). The SAS-related shortening of response onsets tended to be more pronounced in rectus femoris than in tibialis anterior (26 versus 13 ms, *startle x muscle* $F_{1,9} = 4.806$; $p = 0.056$; post-hoc t tests, p(TA) = 0.001, p(RF) = 0.001). There was no significant difference in onset latencies between the left and right leg (*leg,* $F_{1,9} = 2.524$, $p = 0.147$; Figure 6). With regard to the EMG amplitudes, the SAS increased the size of the responses in tibialis anterior and rectus femoris by on average 25% (*startle,* $F_{1,9} = 7.545$, $p = 0.023$; Figure 7). We observed no anticipatory postural adjustments during low-magnitude perturbations.

The effects of a SAS in expected high-magnitude backward perturbations

In contrast to the low-magnitude perturbations, high-magnitude perturbations required participants to take one or more steps to recover balance. Before a step was taken, subjects showed symmetrical response onsets in tibialis anterior and rectus femoris (*leg,* $F_{1,10} = 1.599$, $p = 0.235$), but in addition, consistent activation was also observed

Figure 6 Mean onset latencies (SE) of tibialis anterior (TA) and rectus femoris (RF) in the stance and stepping leg during expected backward perturbations. Dotted lines = low magnitude perturbations. Solid lines = high magnitude perturbations. * indicates significant differences.

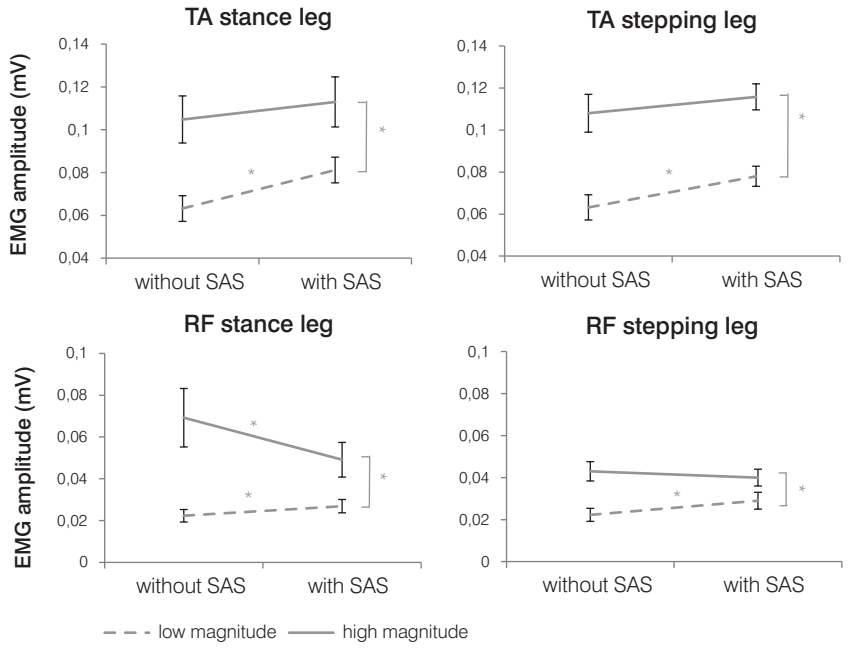


in the biceps femoris of the stepping leg (at 208 ± 35 ms after perturbation onset). The effect of the SAS on onset latencies was similar to the low-magnitude perturbations, again with an even more pronounced acceleration in rectus femoris than in tibialis anterior (31 versus 12 ms, *startle x muscle* $F_{1,10} = 23.722$, $p<0.001$; post-hoc t tests, p(TA)<0.001, p(RF) <0.001; Figure 6, Table 1). A SAS also reduced the onset of the biceps femoris, as the prime mover of the step, by on average 18 ms (*startle*, $p = 0.012$; Table 1).

The SAS did not influence the EMG amplitudes in tibialis anterior, but did result in smaller amplitudes in rectus femoris (*startle x muscle*, $F_{1,10} = 7.455$, $p = 0.021$; post-hoc t tests, p(TA) = 0.242, p(RF) = 0.033; Figure 7). The EMG amplitudes of the rectus femoris were significantly larger in the stance leg than in the stepping leg (*muscle x leg,* $F_{1,10} = 7.612$, $p = 0.020$), but the size of these responses was not differentially modified by the SAS (*startle x muscle x leg*; $F_{1,10} = 2.334$, p=0.158).

The onset of the step was earlier with SAS (283 ±56 ms) than without SAS (293 ± 47 ms), but this difference was not significant (p = 0.093, Table 1). Anticipatory postural

Figure 7 Mean EMG amplitude (SE) of tibialis anterior (TA) and rectus femoris (RF) in the stance and stepping leg during expected backward perturbations. Dotted lines = low magnitude perturbations. Solid lines = high magnitude perturbations. * indicates significant differences.



adjustments were more frequently observed in trials with a SAS (48%) than without (23%, p = 0.021). Six persons showed APAs both with and without a SAS in at least one trial. For those subjects, the onset of the APA was shortened by the SAS (128 ± 36 ms versus 171 ± 34 ms, p = 0.002), without a significant difference in the magnitude of the APA between SAS and no-SAS trials (8 ± 3% versus 7 ± 2% bodyweight, p = 0.368),

Low versus high-magnitude backward perturbations

The observed decrease in tibialis anterior onsets with increasing perturbation magnitude (see above) was similarly present in rectus femoris (13 ms, *magnitude,* $F_{1,10}$ = 36.002, p<0.001, post-hoc t test, p(RF)<0.001; Figure 6, Table 1). Larger perturbations also resulted in increased EMG amplitudes, similarly for tibialis anterior and rectus femoris (*magnitude*, $F_{1,9}$ = 25.042, p = 0.001). The effect of the SAS on the size of the response, however, was only present in low-magnitude perturbations (26% vs -2%, *startle x magnitude,* $F_{1,9}$ = 9.493, p = 0.013, post-hoc t test p(low)=0.023, p(high)=0.745).

The effect of prior knowledge of perturbation direction on SAS-related effects during backward perturbations

The aforementioned SAS-related acceleration of onset latencies, increase in EMG amplitudes and decrease in backward trunk displacement were all independent of prior knowledge of perturbation direction (all *expectation* main and interaction effects, p > 0.105; Table 1). Anticipatory postural adjustments were more often seen with than without advance knowledge of perturbation direction (*expectation,* $F_{1,10}$ = 9.225, p = 0.013), but again without differential effects of the SAS (*expectation x startle,* $F_{1,10}$ = 0.753, p = 0.406, Table 3).

Table 3 Incidence of APAs during high-magnitude perturbations.

	Without SAS	With SAS
Direction known	23%	48%
Direction unknown	12%	30%

Effects of the SAS on tibialis anterior activation during forward perturbations

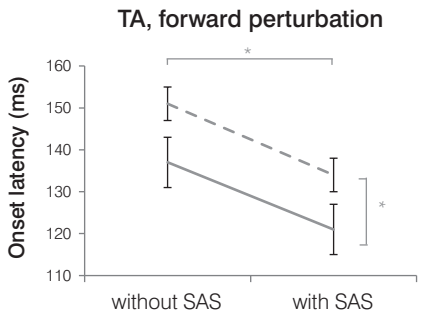
As we observed fairly consistent muscle activity in tibialis anterior during forward perturbations, we determined for each individual trial whether the tibial anterior EMG activity rose above a threshold of 2 SD above the mean background activity, as calculated over a 100 ms period just prior to perturbation onset. We looked for tibialis anterior onsets within the same time interval in which they occurred during backward perturbations (until 160 ms after perturbation).

In all but one participant, muscle onsets were detected in tibialis anterior in one or more forward perturbations. The SAS not only resulted in a higher probability of tibialis anterior responses *(startle,* $F_{1,10}$ = 5.419; p = 0.047; Table 4), but also shortened the onset latencies by 17 ms (*startle,* $F_{1,9}$ = 21.058; p = 0.001; Figure 8, Table 1). These effects were again independent of prior knowledge of perturbation direction (*expectation,* $F_{1,10}$ = 0.101; p = 0.757 and $F_{1,9}$ = 1.014; p = 0.340, respectively). Tibialis anterior onsets were observed earlier (-14 ms) and more frequently (+ 19%) in high than in low-magnitude perturbations (*magnitude,* $F_{1,9}$= 20.722 ; p = 0.001 and $F_{1,10}$ = 8.046; p = 0.018, respectively). We observed no sequence effects within one condition. No consistent activity in rectus femoris was seen during the forward perturbations.

Table 4 Probability of occurrence of onset latencies in tibialis anterior during forward perturbations.

	Low-magnitude		High-magnitude	
	Direction known	Direction unknown	Direction known	Direction unknown
With SAS	65%	65%	76%	77%
Without SAS	43%	50%	72%	72%

Figure 8 Mean onset latencies (SE) of tibialis anterior (TA) during forward perturbations. Dotted lines = low magnitude perturbations. Solid lines = high magnitude perturbations. * indicates significant differences.



Startle reflexes in sternocleidomastoid

Auditory startle reflexes in the sternocleidomastoid muscle were identified in all subjects (at an average onset of 72 ± 25 ms). The occurrence of startle reflexes was infrequent (29 % of trials) and rates of occurrence were not different between the conditions ($F_{1,9}<0.926$; $p>0.361$). The acceleration of latencies by the SAS was independent of reflex activity in the sternocleidomastoid muscle (see Figure 2 for acceleration without startle reflexes). In addition, during 'SAS-only' trials, responses in leg muscles occurred both in trials with and without sternocleidomastoid reflex activity.

Discussion

We investigated the possible interaction between startle and postural responses. The main findings were that a SAS given at the start of a backward directed balance perturbation accelerated the onset of the subsequent corrective postural responses (in tibialis anterior, rectus and biceps femoris) and increased their amplitude, irrespective of whether the perturbation direction was known in advance or not. During forward perturbations, a concurrent SAS did not shorten the onset latency of the prime mover (gastrocnemius), but responses in tibialis anterior were more prevalent and occurred at shorter latencies compared to perturbations without SAS.

The acceleration of responses when a SAS is delivered simultaneously with the imperative cue is known as the 'StartReact' phenomenon.[355,356] This was first demonstrated in experiments involving ballistic voluntary arm movements. More recent experiments have shown that more complex whole-body movements – such as sit-to-stance transfers, stepping and reactions to avoid sudden obstacles – can also be accelerated by a SAS.[209,293,300] In addition to the acceleration of movements, a SAS is also able to strengthen the response.[198,293] The present study is the first to show that postural responses to backward perturbations can be accelerated and strengthened by a SAS. Importantly, our results indicated that the acceleration and strengthening of the postural response with a SAS was beneficial for maintenance of upright balance, as we recorded a 15% smaller backward displacement of the trunk during 'feet-in-place' balance recovery. Furthermore, the SAS increased the incidence of anticipatory postural adjustments and tended to accelerate the step onset, and both of these effects were also beneficial for balance recovery.

Interestingly, previous research demonstrated that a SAS can also trigger the requested movement at similarly short onset latencies when applied in the absence of the imperative signal.[198,293,357] In our experiment, when the SAS was applied without balance perturbation, the muscle responses recorded in tibialis anterior were observed at latencies similar to those during backward perturbations with a SAS. This is in line with recent work of Campbell and co-workers, who found that a SAS can trigger a postural response in the absence of a balance perturbation.[51]

Previous work suggested that the StartReact effect would be the greatest when accompanied by a startle reflex in the sternocleidomastoid muscle.[57] However, in line with other studies, we report that the SAS is able to trigger rapid responses in the absence of SCM responses as well.[209,300,349]

The mechanism underlying the startle-induced effects on postural responses

The precise mechanism underlying the StartReact phenomenon remains unclear. However, there is converging evidence that movements can be encoded and 'stored' in a pre-prepared state in the reticular formation where they are subject to triggered reflex-like release, such as a SAS.[55,356] This hypothesis is supported by numerous studies involving visually triggered movements that are driven by corticospinal output. In contrast, in the present study we investigated medium latency postural responses that are mediated by group II or group Ib afferents, and that do not involve transcortical pathways.[176,292,348] Animal studies have shown that these responses are likely encoded by assemblies of neurons in the pontomedullary reticular formation (pmRF), which synapse onto spinal interneurons as far as the lumbar level.[337] As the pmRF is also involved as a relay station in the startle neural circuit, [194,386] it may be that pmRF neurons involved in postural response generation are also responsive to auditory startling input, but this suggestion is speculative and requires further investigation.

It may be argued that the combination of two stimuli (auditory and proprioceptive) may have strengthened the input to the postural control system, which is usually referred to as intersensory facilitation.[255] This mechanism has previously been demonstrated to enhance the amplitudes of obstacle avoidance responses in combination with a SAS[293] and we also observed increased amplitudes with SAS in perturbations of low magnitude. It is unlikely to explain the shortening in response latencies, however, as we found that a SAS yielded similar tibialis latencies both with and without the administration of a balance perturbation. Furthermore, intersensory facilitation would not likely pertain exclusively to responses in only one direction of perturbation, as observed in the present study.

Differential effects of the startle in forward and backward perturbations

Interestingly, postural responses in the gastrocnemius following forward perturbations were not accelerated by the SAS, irrespective of the expectedness of perturbation direction. In fact, a SAS resulted in more common responses in tibialis anterior at reduced onset latencies. In addition, during 'SAS-only' trials, muscle responses were more often seen in tibialis anterior and rectus femoris compared to gastrocnemius. For tibialis anterior, this was even true when forward perturbations were anticipated. These unidirectional effects of a SAS are unprecedented in StartReact research. For ballistic wrist and elbow movements, shorter onsets latencies were observed with a SAS in both flexion and extension directions.[54,162] Furthermore, step adjustments in response to an obstacle or a mid-step target jump were also similarly accelerated in

either direction.[293,300] In contrast, our results indicate that a SAS particularly accelerates and strengthens responses that protect against backward balance loss. Moreover, the presently observed effects of the SAS on tibialis anterior responses during forward perturbations suggest that these may also represent postural responses normally coinciding with backward balance perturbations, which can even be triggered when a forward balance perturbation is expected.

Both the SAS and the perturbation magnitude exerted very consistent differential effects on the postural responses elicited by backward and forward perturbations. Previous studies also reported on differences between forward and backward postural responses. Unilateral leg perturbations elicited bilateral similar responses in the tibialis anterior during backward perturbations, whereas during unilateral forward perturbations the activity of the gastrocnemius muscle in the non-displaced leg was less than that in the displaced leg.[93] Furthermore, task- and context-related changes were more clearly present in the tibialis anterior muscle than in the soleus muscle.[318] Finally, studies on development of postural control in children demonstrated that complex direction-specific postural responses are present before an infant is able to sit without help,[158] but that the synergy of the dorsal muscles appears to mature earlier than that of the synergy of the ventral flexors.[127,147] These findings raise the question whether postural responses in either direction involve different neural circuits,[93,95] with startle circuits selectively interacting with postural responses to recover from backward perturbations.

The functional role of this suggested neural organization remains speculative, but it may be that any (startling) perturbation to upright balance invariably elicits a default postural response protecting against balance loss in the most unstable (backward) direction. This may also explain why similar tibialis and rectus responses, and similar SAS-effects thereon, were observed irrespective of prior knowledge of perturbation direction. Further downstream, the output to the peripheral muscles may then be 'shaped' by the available sensory input detailing the perturbation characteristics (e.g. direction). This might explain the relatively infrequent occurrence and low amplitudes of responses in SAS-only trials, as they seem to be gated out completely or abolished quickly after onset in the absence of afferent information signalling a perturbation. This modulation presumably takes place at the level of the spinal interneurons, as these receive converging input from both reticular neurons and from peripheral afferents.[316]

Future perspectives

The possibility that startle pathways are involved in postural responses to backward perturbations may have important clinical implications, as patients with Parkinson's disease (PD) and progressive supranuclear palsy (PSP) predominantly experience postural instability and falls in the backward direction.[62,100] Interestingly, these patients (in particular those with PSP, and likely PD-patients with postural instability) also have absent or reduced startle reflexes.[135,195,349,367] As the pedunculopontine nucleus (PPN) is the main structure governing the primary startle circuit[174,193,298] and its function is affected in PD and PSP, patients with implanted PPN stimulators provide a unique study population to investigate the presumed interaction between startle and motor circuits in the pmRF. Recently, Thevathasan and co-workers indeed demonstrated that for arm movements, the StartReact phenomenon was absent without, but present with PPN stimulation ON.[349] In line with the presently suggested involvement of startle pathways in postural responses, these patients also benefited from PPN stimulation with regard to their scores on the gait and falls questionnaire. It remains for future study to more directly assess the relationship between defective postural control and reduced sensitivity to auditory startles in these patients.

Conclusion

We conclude that postural responses to backward and forward perturbations are probably processed by different neural circuits, with a selective involvement of startle pathways in postural responses to backward perturbations. Our results give directions for future studies investigating whether deficits in startle pathways may explain the prominent backward postural instability in patients with Parkinson's disease and PSP.

4

Subcortical structures in humans can be facilitated by transcranial direct current stimulation

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Abstract

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that alters cortical excitability. Interestingly, in recent animal studies facilitatory effects of tDCS have also been observed on subcortical structures. Here, we sought to provide evidence for the potential of tDCS to facilitate subcortical structures in humans as well. Subjects received anodal-tDCS and sham-tDCS on two separate testing days in a counterbalanced order. After stimulation, we assessed the effect of tDCS on two responses that arise from subcortical structures; (1) wrist and ankle responses to an imperative stimulus combined with a startling acoustic stimulus (SAS), and (2) automatic postural responses to external balance perturbations with and without a concurrent SAS. During all tasks, response onsets were significantly faster following anodal-tDCS compared to sham-tDCS, both in trials with and without a SAS. The effect of tDCS was similar for the dominant and non-dominant leg. The SAS accelerated the onsets of ankle and wrist movements and the responses to backward, but not forward perturbations. The faster onsets of SAS-induced wrist and ankle movements and automatic postural responses following stimulation provide strong evidence that, in humans, subcortical structures - in particular the reticular formation - can be facilitated by tDCS. This effect may be explained by two mechanisms that are not mutually exclusive. First, subcortical facilitation may have resulted from enhanced cortico-reticular drive. Second, the applied current may have directly stimulated the reticular formation. Strengthening reticulospinal output by tDCS may be of interest to neurorehabilitation, as there is evidence for reticulospinal compensation after corticospinal lesions.

Introduction

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that alters cortical excitability via application of a weak direct current. The proposed neuronal mechanism underlying the observed facilitatory or inhibitory effects on cortical output involves slight shifts in the resting membrane potential of cortical neurons.[19,78,291] In humans, facilitation of cortical areas by means of anodal tDCS has been found to improve several motor and cognitive functions,[44,263] which has sparked a wealth of research on its utility in patients with central injuries. Interestingly, a recent study in anaesthetized cats showed that tDCS not only affects cortical excitability, but also facilitates subcortical neurons.[37] Rubrospinal and reticulospinal neurons were facilitated by anodal tDCS over the sensorimotor cortex, resulting in shortened latencies and/or increased amplitudes of descending volleys. Such remote effects of tDCS may greatly expand its potential utility in patients with central injuries, but whether tDCS can also facilitate subcortical structures in humans is yet unknown. An imaging study has suggested that subcortical facilitation by tDCS may indeed be possible in humans,[201] but direct evidence is lacking. In this study, we sought to provide evidence for the potential of tDCS to facilitate subcortical structures in humans. We established the effect of tDCS in healthy human subjects on responses that originate from subcortical structures. First, we examined wrist and ankle responses to an imperative 'go' signal, both with and without simultaneous presentation of a startling acoustic stimulus (SAS). A SAS accelerates the onset latencies of movement responses,[356] which has been termed 'StartReact effect'. The shortened onset latencies reflect a direct subcortical release of motor programs.[55,271,359] Second, we examined automatic postural responses to external balance perturbations, with and without a concurrent SAS. These initial postural responses, both with and without a SAS, also arise from subcortical structures.[176]

Materials and methods

Participants

Ten healthy adults (4 women, mean 22 years, range 18-27) participated in this study. Nine participants were right-handed as verified by the Edinburgh Handedness Inventory.[277] These nine participants also showed dominance of the right leg, as identified by the question 'with which foot would you kick a soccer ball'? None of the participants suffered from hearing, neurological or motor disorders that could interfere with performance during the experiments. The study was approved by the local medical ethics committee (CMO region Arnhem/Nijmegen) and was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent prior to the experiment.

Experimental setup and protocol

Participants were measured on two different measurement sessions (separated by at least one week) in which they first received 'anodal-tDCS' or 'sham-tDCS'. The order of the stimulation type was counterbalanced across subjects. Following stimulation, simple reaction times (wrist flexion and ankle dorsiflexion) and onset latencies of postural responses were evaluated. The order of the tasks was also counterbalanced across subjects.

We designed our protocol such that the assessments could be completed within 30 minutes after stimulation because of the time-limited effect of tDCS.[263] Due to these time limitations, we chose to assess wrist flexion unilaterally (ipsilateral to the hemisphere receiving anodal stimulation), in light of the evidence that arm flexors predominantly receive ipsilateral reticulospinal projections.[108] Ankle dorsiflexion was assessed bilaterally, as it is yet unknown whether reticulospinal projections to the dorsiflexor muscles are predominantly ipsilateral or contralateral.

tDCS. tDCS was applied by a battery-driven constant-current stimulator (DC-STIMULATOR PLUS, NeuroConn, Illmenau, Germany) via conductive-rubber electrodes, placed in two saline-soaked sponges (5x7 cm). The anodal electrode was placed over the non-dominant motor region (C3/C4 on the 10-20 international electroencephalogram system). The reference electrode was placed over the contralateral supraorbital region. We stimulated the non-dominant motor region, as we evaluated wrist flexion in the dominant arm, with the arm flexors receiving dominant ipsilateral cortico-reticular projections.[108] During anodal stimulation, tDCS was applied for 15 minutes at an intensity of 2 mA. The current was ramped up to its target intensity over 10 seconds and ramped down in the same time interval at the end of the stimulation period. During sham stimulation, the same procedure was followed but current was applied for 15 seconds only after the first ramp period, followed by 10 seconds ramp down. Stimulation was applied in standing position. Two participants were able to differentiate between the sham and anodal condition, whereas the remaining eight participants could not indicate which session involved anodal-tDCS.

Simple reaction time task. Participants sat in a chair with their hip, knee and ankle joints in 90 degrees. The chair was positioned 2.5 meters in front of two arrays of light-emitting diodes (LEDs; 11x8 cm, 3 cm apart). Illumination of the first LED array formed a warning signal. We instructed participants to respond as rapidly as possible to illumination of the second LED array (i.e., imperative stimulus) in three separate movement tasks; 1) dorsiflexion of the dominant or 2) non-dominant ankle, or 3) flexion of the dominant wrist. The order of the conditions was counterbalanced across subjects. Warning periods (1-3.5 seconds) and inter-trial periods (6-10 seconds) were variable. In each condition, participants performed 16 trials. In 25% of trials a startling

acoustic stimulus (SAS) was given simultaneously with the imperative stimulus. The SAS was given through binaural earphones and consisted of 50 ms white noise with an intensity of 116 dB (sound pressure level), and was generated by a custom-made noise generator.

For the condition involving wrist flexion, the participant's arm was secured in a semi-prone position with the palm facing inward to a custom-made wrist manipulandum that moved in the transverse plane with an axis of rotation at the wrist joint.[54,271]

Automatic postural responses. Participants stood on a moveable platform that could suddenly and unexpectedly translate in the forward or backward direction.[264] A forward translation of the platform resulted in a backward balance perturbation and vice versa. In the remainder of this text, we will refer to the direction of the balance perturbation. Platform movements comprised an acceleration phase (300 ms), a constant-velocity phase (500 ms) and a deceleration phase (300 ms). Both forward and backward perturbations were delivered by platform acceleration of 0.75m/s^2. Participants received 16 forward and 16 backward balance perturbations in a random order. In 25% of both forward and backward trials, the perturbation was combined with a SAS that was administered through binaural earphones at the start of the platform movement. Consecutive trials were separated by at least 20 seconds. On both testing days, subjects received four practice trials before tDCS (two for each direction). Participants were instructed to sustain the perturbations without taking a step or grabbing the handrails surrounding the platform for support.

Data collection

Muscle activity was measured using surface electromyography (EMG) data from bilateral tibialis anterior and gastrocnemius medialis muscles and from the dominant flexor carpi radialis muscle (ZeroWire by Aurion, Italy; 2000 Hz). Self-adhesive Ag-AgCl electrodes (Tyco Arbo ECG) were placed approximately 2 cm apart and longitudinally on the belly of each muscle, according to Seniam guidelines. [156] EMG signals were sampled at 2000 Hz and full-wave rectified and low-pass filtered at 30 Hz (zero-lag, second order Butterworth filter). Furthermore, to assess movement onset, a triaxial accelerometer was placed at the foot or hand involved in the simple reaction task. Accelerometer signals were sampled at 2000 Hz.

Data analysis

Simple reaction time task. Two reaction time parameters were assessed: EMG reaction time and accelerometer reaction time. For each condition, we calculated ensemble average EMG and accelerometer traces, separately for trials with and without a SAS. Onset latencies of the muscles of interest were determined using a semi-automatic computer algorithm that selected the first instant at which the mean EMG activity

exceeded a threshold of 2 standard deviations (SD) above the mean background activity, as calculated over a 500 ms period just prior to the imperative 'go' signal. Onsets were first selected by the computer algorithm, then visually approved and (when necessary) corrected.[293] Average onset latencies were calculated separately for trials with and without a SAS. The onset of foot and wrist acceleration was determined in the same manner.

Automatic postural responses. We determined the latencies of the prime movers of the postural responses using the algorithm described above. For forward perturbations, we identified the onset latencies in the gastrocnemius medialis muscle; for backward perturbations, we determined the onset latencies in the tibialis anterior muscle.

Statistical analysis

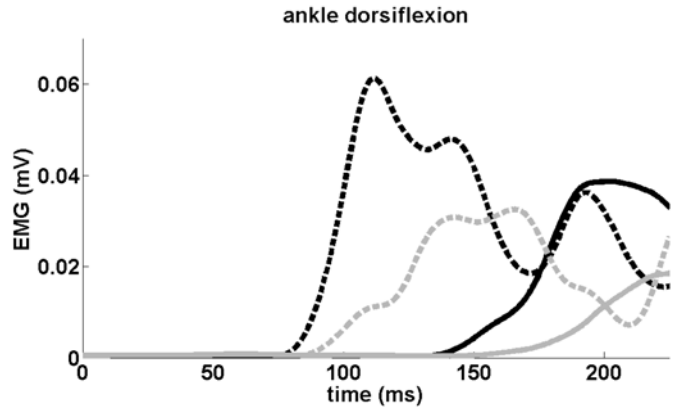
Ankle dorsiflexion reaction times and latencies of automatic postural responses were evaluated using a repeated measures ANOVA, with SAS (*SAS – no SAS*), tDCS (*anodal-tDCS – sham-tDCS*) and leg (*dominant – non-dominant*) as within subjects factors. Wrist flexion reaction times were evaluated using SAS and tDCS as within subjects factors. Main effects are reported as well as *SAS x tDCS* effects. Other interaction effects are only reported if significant. The alpha level was set at 0.05.

Results

Simple reaction time tasks

The EMG traces of a representative subject during ankle dorsiflexion are shown in Figure 1. Group latencies were significantly shorter after anodal-tDCS compared to sham-tDCS (7 ms shortening; *tDCS*; $F_{1,9}$=13.840; p=0.005), which effect was observed irrespective of whether or not a SAS was given (*tDCS x SAS*; $F_{1,9}$=0.181, p=0.681, see Figure 2). A SAS significantly accelerated the onset latency of the tibialis anterior muscle, both following anodal-tDCS (51 ms acceleration) and sham-tDCS (52 ms acceleration; *SAS*; $F_{1,9}$=126.642, p<0.001). Onset latencies and tDCS effects did not differ between the dominant and non-dominant leg (*leg;* $F_{1,9}$=0.859 p=0.378). The same pattern of results was obtained from the accelerometer onsets. Latencies were shorter after anodal-tDCS compared to sham-tDCS (9 ms shortening; *tDCS*; $F_{1,9}$=0.327, p=0.028, see Figure 2), which effect was not differentially affected by the presence of a SAS (*tDCS x SAS*; $F_{1,9}$=0.002, p=0.968). Latencies were significantly accelerated by the SAS, both following anodal-tDCS (57 ms acceleration) and sham-tDCS (57 ms acceleration, *SAS*; $F_{1,9}$=225.406 p<0.001). Again, we found no differences between the dominant and non-dominant leg (*leg;* $F_{1,9}$=1.076, p=0.327).

Figure 1 EMG signals of a representative subject from the tibialis anterior muscle during ankle dorsiflexion with the dominant leg. Grey lines represent trials after sham-tDCS, black lines after anodal-tDCS. Dotted lines represent trials with a SAS, solid lines trials without a SAS.



During wrist flexion, latencies of the flexor carpi radialis muscles were shorter after anodal-tDCS compared to sham-tDCS (12 ms shortening; *tDCS*; $F_{1,9}$=7.306, p=0.024, see Figure 2), which effect was observed irrespective of whether or not a SAS was given (*tDCS x SAS*; $F_{1,9}$=0.032, p=0.868). The SAS accelerated the onset latencies, both following anodal-tDCS (58 ms acceleration) and sham-tDCS (59 ms acceleration; *SAS*; $F_{1,9}$=56.416, p<0.001). This pattern was confirmed by the accelerometer data; latencies were shortened after anodal tDCS compared to sham-tDCS (10 ms shortening; *tDCS*; $F_{1,9}$=7.120, p=0.026), which effect was observed irrespective of whether or not a SAS was given (*tDCS x SAS*; $F_{1,9}$=1.558, p=0.243). A SAS accelerated the onset latencies following both anodal tDCS (71 ms acceleration) and sham tDCS (65 ms acceleration; *SAS*; $F_{1,9}$=155.007, p<0.001).

Postural responses

Onsets of tibialis anterior responses to backward balance perturbations were faster following anodal-tDCS compared to sham-tDCS (7 ms shortening; *tDCS*; $F_{1,9}$=5.398, p=0.045; see Figure 3), which effect was not differentially affected by the presence of a SAS (*tDCS x SAS*; $F_{1,9}$=2.408, p=0.155). A SAS significantly accelerated response onsets to backward balance perturbations, both following anodal-tDCS (15 ms acceleration) and sham-tDCS (10 ms acceleration; *SAS*; $F_{1,9}$=6.312, p=0.033). There were no differences between the dominant and non-dominant leg (*leg*; $F_{1,9}$=0.852, p=0.380).

Gastrocnemius responses to forward perturbations were on average 10 ms faster after anodal-tDCS compared to sham-tDCS (*tDCS*; $F_{1,9}$=8.484, p=0.017, see Figure 3).

Figure 2 Mean onset latencies (SE) during the simple reaction time tasks involving voluntary ankle dorsiflexion and wrist flexion. * significant difference between trials with and without a SAS (main effect). + significant difference between anodal-tDCS and sham-tDCS (main effect).

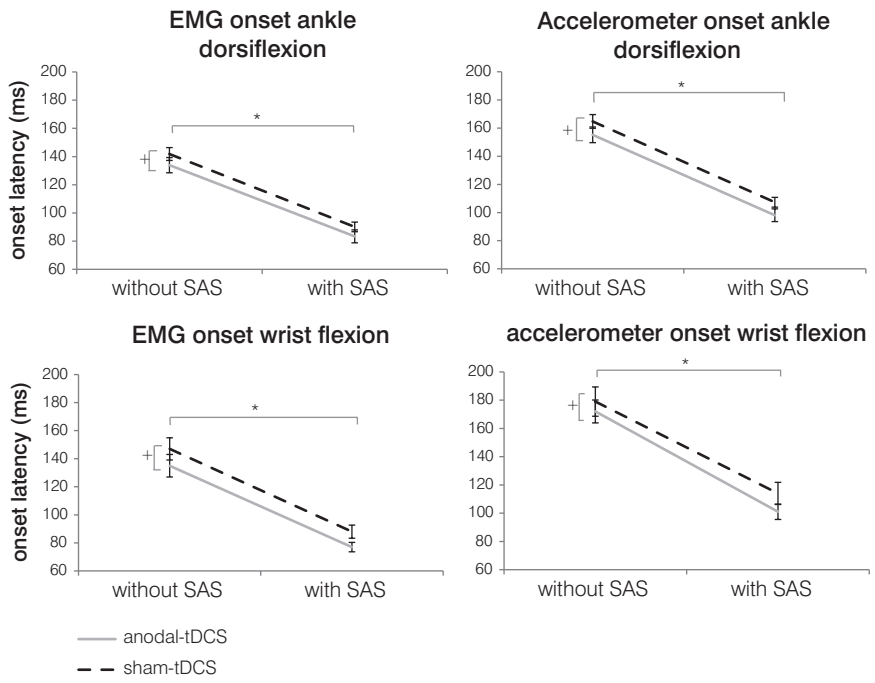
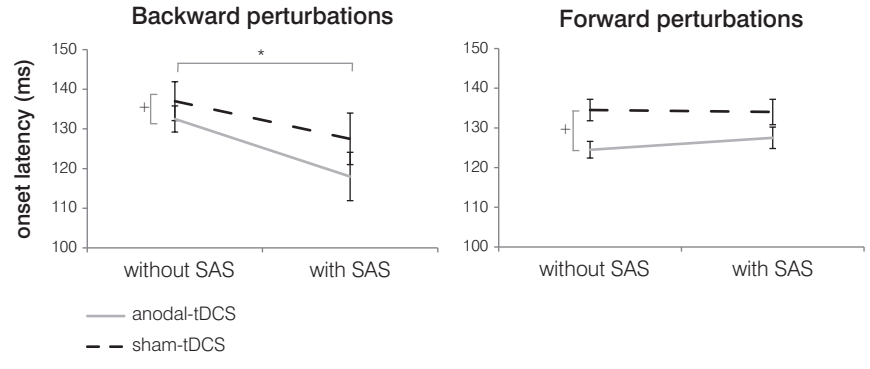


Figure 3 Mean onset latencies of prime movers of postural responses (SE) to backward (tibialis anterior muscle) and forward (gastrocnemius medialis muscle) perturbations. *significant difference between trials with and without a SAS (main effect). +significant difference between anodal-tDCS and sham-tDCS (main effect).



A SAS did not accelerate gastrocnemius responses to forward perturbations (*SAS*; $F_{1,9}$=0.567, p=0.471). Again, there were no differences between the dominant and non-dominant leg (*leg;* $F_{1,9}$=1.289, p=0.286).

Discussion

In this study we aimed to establish whether transcranial direct current stimulation (tDCS) is able to facilitate subcortical motor responses in humans. We examined the effects of anodal tDCS over the non-dominant motor region on two types of motor responses that originate from subcortical structures, 1) SAS-induced wrist flexion and ankle dorsiflexion movements, and 2) postural responses to forward and backward perturbations, with and without a concurring SAS. In all tasks, responses were significantly shorter after anodal-tDCS compared to sham-tDCS, both in trials with and without a SAS. For ankle dorsiflexion as well as postural responses, the effect of tDCS did not differ between the dominant and non-dominant leg. These results support the hypothesis that tDCS facilitates not only cortical, but also subcortical structures.

Subcortical origin of StartReact and postural responses

For the interpretation of our results, it is important to highlight the evidence for the subcortical origin of the responses studied. The origin of SAS-induced responses is a matter of an ongoing debate, but a recent study provided strong evidence that subcortical structures, in particular the reticular formation, play a key role in the StartReact effect.[271] Three hypothesis have been proposed to explain the StartReact effect. The first and prevailing hypothesis is a direct release of a subcortically stored motor program by the SAS,[55,356] conveyed by the reticulospinal tract.[310,359] The second hypothesis proposes that the SAS could act as a subcortically mediated trigger for a cortically stored motor program, conveyed by the corticospinal tract.[3,60] This second hypothesis is supported by the observation that the acceleration of motor responses by a SAS can be delayed by transcranial magnetic stimulation (TMS) over the motor cortex.[3,220] Moreover, a recent study using EEG highlighted the role of cortical pre-motor areas in the preparation of SAS-induced movements.[210] Third, a SAS could act as an additional stimulus on top of the imperative stimulus, thereby increasing the energy of the sensory input, a process known as intersensory facilitation.[255] Intersensory facilitation could subsequently lead to faster sensorimotor coupling at cortical level, resulting in accelerated release of motor programs conveyed by the corticospinal tract. Importantly, SAS-induced responses are likely dissociated from startle reflexes as StartReact is often observed in the absence of standard markers of startle reflexes.[52,209,266,269,300,307] In a recent study we tried to unravel the hypotheses

described above by applying the StartReact paradigm to patients with hereditary spastic paraplegia (HSP).[271] HSP is a disease characterized by retrograde axonal degeneration of the corticospinal tract, while leaving the reticulospinal tract unaffected.[265] Typically, HSP in its pure form does not affect the corticospinal tracts innervating the motoneurons of the upper extremities. In our study, we compared the StartReact effect between a reaction task involving ankle dorsiflexion and a task involving wrist flexion.[271] Simple reaction times of ankle dorsiflexion were delayed in the patients with HSP compared to healthy controls, which coincided with delayed motor evoked potentials in tibialis anterior in response to supramaximal TMS. When the ankle dorsiflexion task was combined with a SAS, however, reaction times in the patients were accelerated to a larger extent than in the controls, resulting in completely normalized EMG and movement onset latencies. When the reaction time task involved voluntary wrist flexion instead of ankle dorsiflexion, no differences in onset latencies between patients and controls were recorded, irrespective of whether a SAS was applied. This pattern of results provides strong evidence for the hypothesis that a SAS accelerates reaction times by releasing a subcortically stored motor program conveyed by the reticulospinal tract.

One might argue that our study in patients with HSP provided evidence for subcortical pathways mediating SAS-induced ankle dorsiflexion responses, but that there is no direct evidence for SAS-induced wrist flexion responses originating from these structures. Yet, in people with hemiparetic stroke, a similar preservation of SAS-induced acceleration of onset latencies in the upper extremity has been demonstrated,[162,164] which suggests that these responses are also conveyed by fast subcortical pathways. Moreover, in healthy humans, StartReact responses in the upper and lower extremities exhibit the same characteristics, since they leave the muscle activation pattern unaffected and show the same degree of SAS-induced acceleration.[356] Hence, the mechanism underlying StartReact effects in the upper and lower extremities is likely the same.

There is strong evidence that, in line with StartReact responses, the reticular formation plays a key role in postural responses as well. In the present study, we investigated medium latency (automatic) postural responses that are mediated by group II or group Ib afferents. These responses have convincingly been shown not to involve transcortical pathways.[176,292,348] Animal studies have demonstrated that, instead, they are likely encoded by neurons in the reticular formation, which synapse onto spinal interneurons.[337]

Acceleration of automatic postural responses

Not only voluntary reaction times, but also automatic postural responses to backward balance perturbations can be accelerated by a SAS.[51,266] In the present study, we

found SAS-induced acceleration of postural responses to backward, but not to forward perturbations. These results mirror those previously reported by our group.[266] It has been hypothesized that postural responses to both forward and backward perturbations are evoked from the reticular formation, but involve different neural circuits[266] with only backward-perturbation response pathways receiving input from startle circuits. However, it remains to be investigated why the SAS-induced acceleration of postural responses is direction specific.

Although the SAS-induced acceleration of postural responses was smaller than the acceleration of voluntary movements, there is evidence that SAS-induced postural responses are consistent with a StartReact effect as well. Previous studies have demonstrated that a SAS can trigger a voluntary movement at similarly short onset latencies when applied in the absence of the imperative signal.[198,293,357] This characteristic of StartReact responses has proved to be applicable to postural responses as well. Two studies have shown that postural responses can be triggered by a SAS in the absence of a balance perturbationf[51,266] with similar latencies to those in the presence of a perturbation.[266] Because of this observation, it is unlikely that the SAS-induced shortening of postural response latencies is due to intersensory facilitation. Furthermore, the observation of unidirectional SAS-induced acceleration of postural responses is also not consistent with intersensory facilitation, as this mechanism would likely accelerate responses to both forward and backward perturbations.

Subcortical structures can be facilitated by tDCS

This study provides evidence for tDCS-induced subcortical facilitation in humans. These findings are in agreement with the recently reported facilitation of reticulospinal and rubrospinal motor neurons by tDCS in anaesthetized cats.[37] Similar has also been reported in rats,[35] albeit evoked by cathodal-stimulation. A previous observation already hinted at tDCS-induced subcortical facilitation in humans, but direct evidence was lacking. It was reported that during and following tDCS there was an increase in regional cerebral blood flow in subcortical structures, including the red nucleus and the mesencephalic and pontine reticular nuclei.[201] This observation may point at an effect of tDCS at the subcortical level, but its functional significance could not be established. The present results demonstrate that tDCS application indeed changed the excitability of subcortical structures, leading to faster response onsets.

The facilitation of subcortical structures by tDCS may be explained by two mechanisms that are not mutually exclusive. First, the subcortical facilitation may have resulted from a change in the cortico-reticular drive. Second, the applied current may have directly changed the excitability of subcortical structures. The latter hypothesis is supported by a modeling study on the spread of current during tDCS application using the same electrode configuration as in this study, which demonstrated the

potential for direct subcortical effects.[371] Both mechanisms were found when tDCS was applied to anaesthetized cats in the study of Bolzoni *et al*.[37]
Alternatively, one might argue that the present results may be explained by an increased arousal or general attention caused by tDCS, which was not present during the sham condition. An increase in arousal or attention could have affected both cortical and subcortical pathways. Indeed, it has shown that tDCS can improve attention and thereby reduces reaction times, likely via facilitation of cortical structures.[136] The effect of attention or general arousal on SAS-induced reaction times has not been investigated, which leaves the possibility of increased general arousal underlying the observed reduction in SAS-induced onset latencies following tDCS. However, this explanation does not seem to hold true for the observed acceleration of postural responses, as there are several studies that suggest that onset latencies of these responses are not influenced by attention or arousal. For instance, responses onsets do not change when attention has to be divided between a postural and a concurrent cognitive task.[274,294] Moreover, in a study that evaluated automatic postural responses to external perturbations in participants while standing in a high postural threat condition, response onsets in the lower extremities were not influenced by anxiety.[63] Hence, it seems unlikely that the acceleration of subcortical motor responses by tDCS as found in the present study can solely be attributed to increased general arousal or attention.

Role of the reticular formation in the StartReact effect and postural responses

The present results raise the question which subcortical structures can be facilitated by tDCS. In the study of Bolzoni et al., tDCS application in anaesthetized cats yielded direct and indirect facilitation of reticulospinal motor neurons.[37] There are several arguments why this may have been the mechanism underlying the present results as well. There is compelling evidence that the pontomedullary reticular formation (pmRF) is critically involved in generating the automatic postural responses to external balance perturbations[161,337] as well as in the StartReact effect.[271] Studies in monkeys and cats have indentified the pmRF as one of the subcortical structures that subserves motor preparation.[45,315] As the pmRF is also a key structure in the startle reflex circuitry,[83,386] it presumably plays a pivotal role in the release of pre-prepared motor programs, resulting in the StartReact effect. Hence, the acceleration of fast SAS-induced ankle and wrist movements and of automatic postural responses following tDCS application over the sensorimotor cortex most likely results from facilitation of the reticular formation. In contrast, responses during reaction time tasks without a SAS most likely originate from the cortex. The tDCS-induced acceleration of ankle and wrist movements during trials without a SAS therefore point to facilitation of cortical structures, which is in line with previous work.[128,173]

Bilateral effects of tDCS

Although the anodal electrode was always positioned over the non-dominant motor region, we found no differences in the effects of tDCS between the dominant and non-dominant leg, both for cortically and subcortically organized responses. One reason may be that the applied current was rather large, which may have resulted in a significant spread of current across the brain also affecting subcortical structures, including cortico-reticular pathways and the brainstem reticular formation. The bilateral effects of tDCS on subcortical structures may thus be explained by a direct (bilateral) effect of tDCS on the reticular formation, or by a change in the cortico-reticular drive. Although cortico-reticular projections are predominantly ipsilateral in humans,[108,388] contralateral projections have also been identified.[183,184]
Responses during reaction time tasks without a SAS, which are mediated by cortical structures, were also bilaterally accelerated by tDCS. This suggests that the direct cortical effects of tDCS were not strictly lateralized either, but the underlying mechanism remains to be investigated. The bilateral effects may also be due to ipsilateral connectivity to lower limb motor neurons.[211] Alternatively, we cannot rule out that bilateral cortical effects resulted from increased arousal evoked by tDCS.

Future studies

The present results have implications for future studies investigating the effects of tDCS on cortically mediated responses. As our study demonstrates that the common application of tDCS over the sensorimotor cortex also yields effects on a subcortical level, the possibility of such effects interacting with the cortical effects of interest should be considered. In addition, future studies may investigate how the subcortical effects of tDCS can be enhanced. Bolzoni *et al.* reported that the facilitation of subcortical structures in anaesthetized cats is enhanced by repeated application of tDCS.[37] We hypothesize that this may also be the case in humans, but this needs to be proven. Furthermore, a modeling study has suggested that the facilitation of ventrally located subcortical structures (i.e., the brainstem) might be larger with the reference electrode placed in contact with the neck muscles (extracephalic position) compared to a supraorbital position.[371] Our paradigm might be useful to study this hypothesis. However, the effects of the extracephalic positioning of the reference electrode should be closely monitored, as a case study reported on disturbed breathing, speech arrest and psychosis after brainstem stimulation.[206,262]

Application in clinical practice

As subcortical structures, in particular the reticular formation, are involved in motor preparation they could play a compensatory role in the recovery after corticospinal lesions.[12] A recent study in monkeys suggested that the reticulospinal tract is indeed responsible for some functional recovery after acute corticospinal lesions, such as

stroke.[387] It has also been suggested that a similar compensatory mechanism may be at work in patients with hereditary spastic paraplegia.[271] Compensation by the reticular formation requires strengthening of the output, not the growth of new neural connections.[12] The application of tDCS may, therefore, be useful to increase the activation of reticulospinal motoneurons or result in a stronger reticulospinal output, both of which could be beneficial for motor recovery and rehabilitation.[37] Interestingly, a recent study in patients with leukoaraoisis (hyperintensities in the subcortical white matter) showed that balance performance improved in response to a combined session of physical training and tDCS over the midline motor and premotor areas, but not following physical training alone.[180] In light of the present results, these improvements may have resulted from tDCS-induced reticulospinal facilitation.

PART 2

Hereditary spastic paraplegia

5

StartReact restores reaction time in HSP: evidence for subcortical release of a motor program

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Abstract

Startling acoustic stimuli (SAS) can accelerate reaction times ('StartReact' effect), but the underlying mechanism remains unclear. Both direct release of a subcortically stored motor program and a subcortically mediated trigger for a cortically stored motor program have been hypothesized. To distinguish between these hypotheses, we examined the StartReact effect in humans with pure hereditary spastic paraplegia (HSP). Delayed reaction times in HSP-patients in trials both with and without a SAS would argue in favor of a cortically stored response.

We instructed 12 HSP-patients and 12 matched controls to respond as rapidly as possible to a visual imperative stimulus, in two different conditions: dorsiflexion of the dominant ankle; or flexion of the dominant wrist. In 25% of trials, a SAS was delivered simultaneously with the imperative stimulus. Prior to these tests, subjects received five SAS while standing to verify normal function of the reticulospinal tract in HSP.

Latencies of startle responses in sternocleidomastoid and tibialis anterior muscles were comparable between patients and controls. During the ankle dorsiflexion task, HSP-patients had an average 19 ms delay in reaction times compared to controls. Administration of a SAS accelerated ankle dorsiflexion in both groups, but more so in the patients, which completely normalized their latencies. The wrist flexion task yielded no differences in onset latencies between HSP-patients and controls.

The reticulospinal tract seems unaffected in HSP-patients, because startle reflex onsets were normal. The corticospinal tract was affected, as reflected by delayed ankle dorsiflexion reaction times. These delayed onsets in HSP were normalized when the imperative stimulus was combined with a SAS, presumably through release of a subcortically stored motor program conveyed by the preserved reticulospinal tract.

Introduction

The startle reflex is an involuntary reaction to unexpected sensory input and is the fastest generalized motor reaction of humans and animals.[359] Auditory startling stimuli can accelerate reaction times when delivered simultaneously with an imperative cue, a phenomenon known as 'StartReact'.[356] The underlying mechanism of this phenomenon is not completely clear.[322] One hypothesis to explain the StartReact effect is that a startling acoustic stimulus (SAS) acts as an additional stimulus on top of the imperative stimulus and thereby increases the energy of the sensory input, resulting in an acceleration of sensorimotor coupling. This is known as intersensory facilitation.[255] The other and prevailing hypothesis for the StartReact effect is a direct release of a subcortically stored motor program by the SAS,[55,356] conveyed by the reticulospinal tract.[310,359] This hypothesis assumes that during motor preparation, motor programs become represented in subcortical structures,[45,315] which are then accessible to startle pathways. Yet, it has recently been proposed that the SAS could also act as a subcortically mediated trigger for a cortically stored motor program.[3,60] This notion would imply that the cortically stored response is triggered without the usual cortical processing, and is conveyed by the corticospinal tract.

To distinguish between the abovementioned hypotheses, we have examined the StartReact effect in patients with a pure form of hereditary spastic paraplegia (HSP). HSP is a diverse group of inherited disorders that are clinically characterized by progressive spasticity, muscle weakness and reduced proprioception of the lower extremities.[311] The common pathological feature of these conditions is retrograde axonal degeneration of the corticospinal tract and the posterior spinal columns, without cortical pathology.[228] The retrograde axonal degeneration is presumably due to abnormal axonal membrane trafficking processes, which primarily affect the distal parts of axons.[22] Using transcranial magnetic stimulation, degeneration of the corticospinal tract in HSP is reflected by prolonged central motor conduction times, elevated cortical motor thresholds, and reduced amplitudes of motor evoked potentials in the lower limbs.[38,177,253,281,289,312] In contrast, motor conduction times, cortical motor thresholds and amplitudes of motor evoked potentials to the arm muscles have been reported to be normal.[177,281,289,312] In HSP, the reticulospinal tract is generally assumed not to be affected by the retrograde degenerative process, but this has not been proven. Therefore, we first examined startle reflex latencies to verify the intact function of the reticulospinal tract in HSP.

Then, to differentiate between the hypotheses explaining the StartReact phenomenon, we used a simple reaction time paradigm involving either voluntary ankle dorsiflexion or voluntary wrist flexion. The retrograde corticospinal axonal degeneration in the patients with HSP was expected to affect responses in the legs, but not in the arms.

For a direct release of a subcortically stored motor program to explain the StartReact effect we expected two findings. First, we expected to observe delayed simple reaction times during dorsiflexion, because of the degenerated corticospinal tract. Second, when the imperative 'go' signal would be combined with a SAS, we expected to see normal latencies as, in this hypothesis, the SAS is able to launch a subcortically stored motor program conveyed by the reticulospinal tract. In contrast, delayed dorsiflexion reaction times both with and without a SAS would argue in favor of a cortically stored response or intersensory facilitation as underlying mechanism of the StartReact phenomenon.

Materials and methods

Participants

Twelve patients with autosomal dominant forms of HSP (9 men; mean age 51 years, range 23-68 years) were recruited from all patients with HSP who were known at the outpatient departments of Neurology and Rehabilitation of our university hospital, a tertiary referral centre for HSP. All 12 patients fulfilled the diagnostic clinical criteria for 'pure' HSP;[311] 8 patients had previously been tested positive for pathogenic SPAST (SPG4) mutations, and 1 patient for a pathogenic KIAA0196 (SPG8) mutation. In the other three patients, mutations in genes most frequently associated with AD-HSP (ATL1, SPAST and/or REEP1) had been excluded. All patients were able to walk independently. In addition, twelve aged-matched healthy controls (7 men, mean 49 years, range 23-65) participated.

Ethics statement

The study was approved by the regional medical ethics committee (CMO Arnhem-Nijmegen) and was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent prior to the experiment.

Clinical assessment

Muscle tone of the triceps surae (TS) and tibialis anterior (TA) muscles was assessed using the Modified Ashworth Scale (0-5), with higher scores indicating more hypertonia.[32] The TS muscles were tested both with the knee flexed and extended. Muscle strength of the TS and the TA was assessed with the Medical Research Council (MRC) scale (0-5), with lower scores indicating less muscle strength.[76] We assessed the deep sensory modalities of the legs by testing the vibration sense at the lateral malleoleus and at the first metatarsophalangeal joint (MTP I) using the semi-quantitative tuning fork (0-8) (Rydel Seiffer, Neurologicals, Poulsbo, Washington), with lower scores indicating more sensory loss.[282] Vibration sense at the ankle and

forefoot was averaged to obtain one value. For all measures, the mean of both legs was determined and used for further analysis (Table 1).

Table 1 Clinical assessment of patients with HSP.

MAS tibialis anterior	0 (range 0-0)
MRC tibialis anterior	5 (range 4-5)
MAS triceps surae	2 (range 1-2) with knee extended; 1 (range 1-2) with knee flexed
MRC triceps surae	5 (range 4.25-5)
Vibration sense	4.5 (range 0.25 – 6.25)

Values are median and range. MAS = Modified Ashworth Scale, MRC = Medical Research Council scale, Vibration sense tested using a semi-quantitative tuning fork (0-8).

Assessment of motor conduction time

The motor conduction time to the dominant TA muscle was assessed in 11 patients before participation in the experiment. These motor conduction times were collected to be used in a parallel longitudinal study in the same patient group. One of the twelve patients did not want to undergo TMS of the lower limbs already at baseline, whereas several others refused to participate in follow-up TMS measurements. Therefore, we were unable to collect motor conduction times to the arm muscles. Magnetic stimulation of the cortex was performed using a double 110 mm cone coil, and for lumbar root stimulation a circular 90 mm coil was used, according to the International Federation of Clinical Neurophysiology (IFCN) guidelines.[308,309] All subjects were stimulated at 100% of the maximum stimulator output. Motor cortex stimulation was assessed with slight voluntary contraction of the TA, whereas spinal root stimulation was assessed at rest. The onset of TA activity after motor cortex stimulation was taken as the total motor conduction time (TMCT). Peripheral motor conduction time (PMCT) was obtained after spinal root stimulation. In one patient with HSP, we were unable to determine the PMCT. Corticospinal motor conduction time (CMCT) was assessed by subtracting the PMCT from the TMCT.

Experimental setup and protocol

First, subjects received five SAS while standing. The SAS were given through binaural earphones and consisted of 50 ms white noise with an intensity of 116 dB (Sound Pressure Level, SPL). The SAS was generated by a custom made noise generator. Second, participants performed a warned simple reaction task. For this test, participants sat in a chair placed in front of two blocks with light-emitting diodes

(LEDs). Illumination of the first LED block formed a warning signal and participants were instructed to perform ankle dorsiflexion with the dominant ankle as soon as the second LED block was lit (i.e., imperative stimulus). Warning periods (1 – 3.5 seconds) and inter-trial periods (6-10 seconds) were variable. Participants performed 20 trials, in 25% of which a SAS was given simultaneously with the imperative stimulus. Third, participants performed another reaction time task where they had to flex the dominant wrist. The participant's arm was secured in a semi-prone position with the palm facing inward, to a custom-made wrist manipulandum that moved in the transverse plane with an axis of rotation at the wrist joint.[54] Again, in 25% of the series of 20 trials a SAS was given simultaneously with the visual 'go' signal.

Data collection

Electromyographic (EMG) data were collected from the dominant TA muscle, dominant gastrocnemius medialis (GM) muscle, the dominant flexor carpi radialis (FCR) and left sternocleidomastoid (SCM) muscles (ZeroWire by Aurion, Italy). EMG signals were sampled at 2000 Hz and full-wave rectified and low-pass filtered at 30 Hz (zero-lag, second order Butterworth filter). The applied filtering technique resulted in systematic, small reduction of the detected latencies (on average 7 ms) for trials both with and without a SAS. Furthermore, a triaxial accelerometer was placed at the dominant foot and hand. Accelerometer data were collected to ensure that the SAS did not only result in shortened EMG onsets, but also in shortened movement onsets. Accelerometer signals were sampled at 2000 Hz and low-pass filtered at 30 Hz (zero-lag, second order Butterworth filter).

Data analysis

Auditory startle reflexes were defined as the presence of a short latency response in the sternocleidomastoid muscle within 100 ms after the SAS.[42,349] The response had to exceed, for at least 20 ms, a threshold of 2 SD above mean background activity, as calculated over a 500 ms period just prior to the SAS. Reflex activity in the TA muscle and FCR had to occur within 120 ms after the SAS to exclude any voluntary component.[41] For every participant we assessed for each muscle whether one or more startle reflexes occurred. For every muscle, the percentage of patients demonstrating at least one startle reflex is reported, in addition to the latency of the first occurring response.

Two reaction time parameters were assessed: EMG reaction time and accelerometer reaction time. Onset latencies of the muscles of interest were determined using a semi-automatic computer algorithm that selected the first instant at which the mean EMG activity exceeded a threshold of 2 SD above the mean background activity, as calculated over a 500 ms period just prior to the imperative 'go' signal. Onsets were

first selected by the computer algorithm, then visually approved and (when necessary) corrected.[62,293] Average EMG onset latencies were calculated separately for trials with and without a SAS. The onset of foot and wrist acceleration was determined in the same manner. For the ankle dorsiflexion task, we also calculated the interval between the EMG activity of the TA and GM by subtracting the TA onset from the GM onset.

Statistical analysis

We tested for differences in onset latencies of startle reflexes between patients with HSP and controls using unpaired t-tests. Differences in the rates of occurrence of startle reflexes between patients and controls were tested using a chi-square test. Reaction time parameters were analyzed using a repeated measures ANOVA, with SAS (SAS-no SAS) as a within subjects factor and group (HSP –controls) as a between subjects factor. The alpha level was set at 0.05. In addition, the 95% confidence interval of the mean difference between patients and controls is presented, both for trials with and without a SAS. The statistical analyses were performed using SAS 9.2 for Windows (SAS Institute Inc, USA) and IBM SPSS Statistics 20 for Windows (SPSS, USA).

Results

Motor conduction times

TMCTs in patients with HSP were on average 35.8±5.1(SD) ms and PMCTs were on average 15.6±1.6 ms, resulting in mean CMCTs of 20.2±5.1 ms (95% CI: 17.1 – 23.3 ms). These CMCT values were significantly delayed compared to reference data obtained in healthy subjects (13.8±1.3 ms; 95% CI: 13.2 – 14.4 ms [312] and 13.8±1.5 ms; 95% CI: 13.4 – 14.2 ms [130]).

Startle reflex

SAS while standing clearly induced startle reflexes in the SCM muscle with similar onset latencies in patients with HSP (52±10 ms, rate of occurrence 75%) and control subjects (52±16 ms, rate of occurrence 92%). Neither onset latencies ($t(18)=0.72$, $p=0.943$) nor rates of occurrence ($\chi^2(1)=1.2$, $p=0.273$) differed between the groups. Startle reflexes were also seen in the wrist flexor, both in patients (75±14 ms, rate of occurrence 67%) and controls (84±20 ms, rate of occurrence 58%). The onset latencies ($t(13)=1.064$, $p=0.307$) and rates of occurrence ($\chi^2(1)=0.178$, $p=0.673$) did not differ between the groups either. In the TA muscle, we also recorded similar onset latencies between patients (87±14 ms, rate of occurrence 50%) and controls (94±17 ms, rate of occurrence 42%). Again, neither onset latencies ($t(9)=0.805$, $p=0.442$) nor rates of occurrence ($\chi^2(1)=0.168$, $p=0.682$) differed between the groups.

Ankle dorsiflexion reaction time

Mean EMG traces of a representative patient with HSP and a control subject during the simple reaction task involving voluntary dorsiflexion of the foot are shown in Figure 1. We found no consistent differences in the EMG activation pattern between patients with HSP and controls. In all participants, an asynchronous activation pattern of the TA and GM was observed both in trials with and without a SAS (Figure 1). The onset latency in the TA was 146±23 ms in patients with HSP compared to 127±15 ms in controls in trials without a SAS (Figure 3).

Figure 1 Mean EMG traces of a representative patient with HSP and control subject during ankle dorsiflexion. Grey lines are trials with a SAS, black lines are trials without a SAS. Determined latencies are presented by a dot.

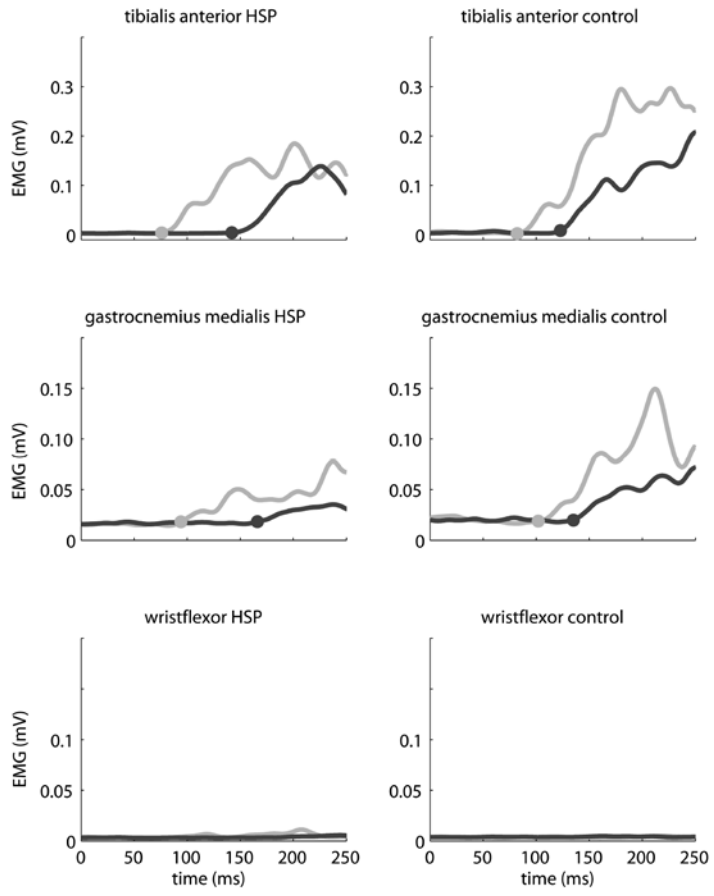
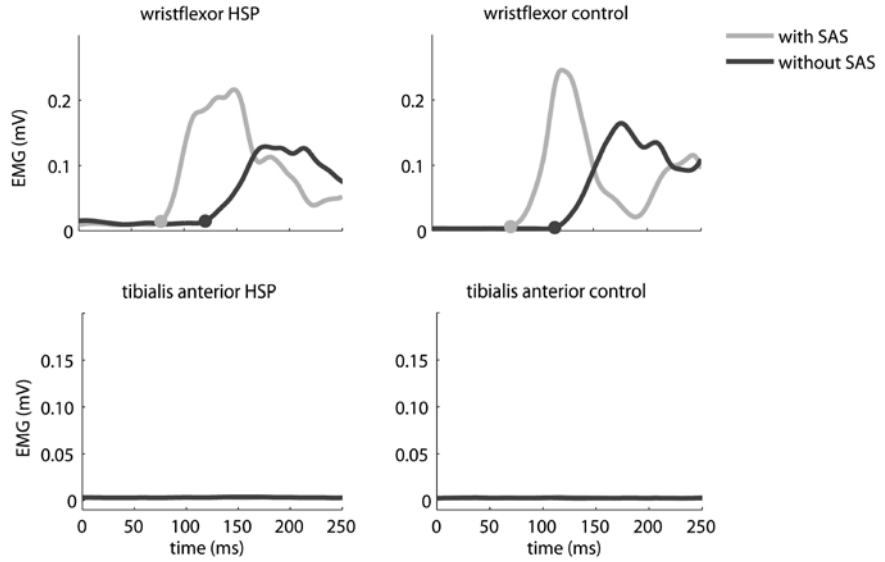


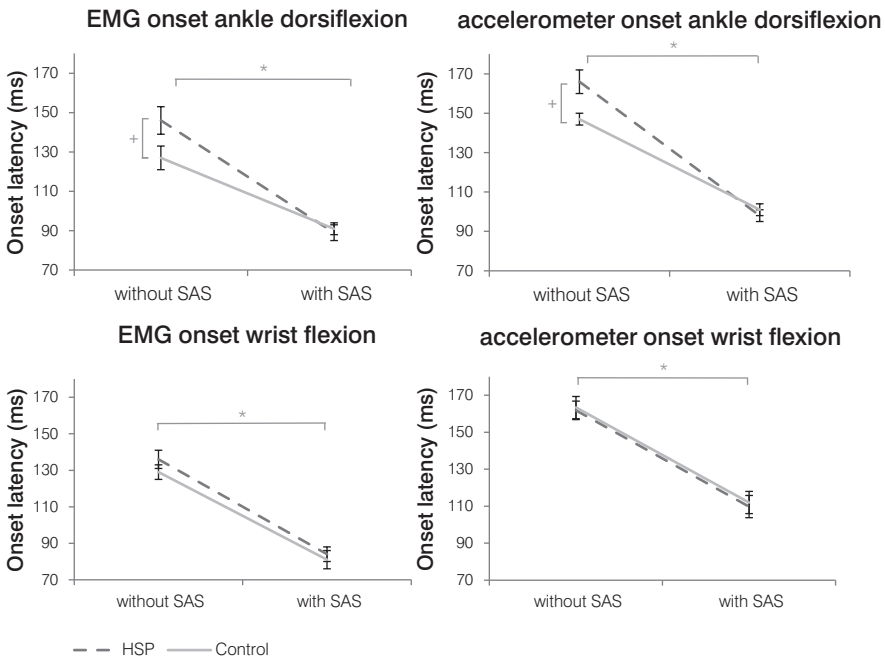
Figure 2 Mean EMG traces of a representative patient with HSP and control subject during wrist flexion. Grey lines are trials with a SAS, black lines are trials without a SAS. Determined latencies are presented by a dot.



Administration of a SAS accelerated ankle dorsiflexion in both groups, but a larger acceleration in HSP patients normalized the latencies (89±20 ms) compared to controls (91±12ms) (SAS; $F_{1,22}$=217.16, p<0,001; SAS x group; $F_{1,22}$=12.028, p=0.002; group; $F_{1,22}$=1.524, p=0.230). Latencies during trials without a SAS were on average 19 ms longer in patients than in controls (95% CI: 4 – 35 ms, p=0.017), but were similar during trials with a SAS (95% CI: -18 – 13 ms, p=0.731). Administration of a SAS did not change the interval between the TA and GM (SAS; $F_{1,22}$=1.792, p=0.194), neither in patients (21±16 ms without SAS vs 17±6 ms with SAS) nor in controls (20±10 ms without SAS vs 17±8 ms with SAS, group; $F_{1,22}$=0.030, p=0.865; SAS x Group; $F_{1,22}$=0.030, p=0.865).

The same pattern of results was seen in the onset latencies as measured with the accelerometer on the foot; in trials without a SAS these were 166±21ms in patients with HSP versus 147±10 ms in controls. A larger SAS-induced acceleration in patient with HSP normalized the latencies (98±20ms) compared to controls (101±9 ms) (SAS; $F_{1,22}$=550.42, p<0,001; SAS x group; $F_{1,22}$=20.493; p<0.001; group; $F_{1,22}$=1.459, p=0.240). Again, latencies during trials without a SAS were on average 19 ms longer in patients with HSP than in controls (95% CI: 5 - 32 ms, p=0.011), but were similar during trials with a SAS (95% CI: -17 - 10 ms, p=0.591).

Figure 3 Mean onset latencies (SE) during the simple reaction time tasks involving voluntary ankle dorsiflexion (upper graphs) and wrist flexion (lower graphs). * indicates significant differences between trials with and without a SAS. + indicates a s significant SAS x group interaction.



Wrist flexion reaction time

Mean EMG traces of a representative patient with HSP and a control subject during the simple reaction task involving wrist flexion are shown in Figure 2. The onset latency of the FCR was accelerated by a SAS, both in patients with HSP (136±17 ms to 84±14 ms) and in control subjects (129±18 ms to 81±20 ms) (SAS; $F_{1,22}$=144.22, p<0.001; Figure 3). There were no differences in the latencies of the FCR responses between the patients and the controls either with or without a SAS (group; $F_{1,22}$=0.652, p=0.428; SAS x group; $F_{1,22}$=0.247, p=0.624). The same pattern was seen when analyzing the onset latencies of the wrist accelerometer data; a SAS accelerated the latencies both in patients with HSP (162±16 ms to 110±14 ms) and in controls (163±22 ms to 111±22 ms) (SAS; $F_{1,22}$=351.964, p<0.001), with no differences between the groups (group; $F_{1,22}$=0.042, p=0.840; SAS x group; $F_{1,22}$<0.001, p=0.988).

Startle reflexes during reaction time tasks

During the ankle dorsiflexion task, 67% of the SAS-trials was accompanied by a startle reflex in the SCM muscle, both in patients with HSP and in controls. During the

wrist flexion task, 60% of the SAS-trials was accompanied by a SCM-reflex in patients with HSP, and 67% of the SAS-trials in controls. When only SAS trials with a concurrent SCM-reflex were analyzed, the effect sizes and levels of significance were the same as when all SAS trials were used for analysis.

Discussion

This study aimed to investigate the mechanisms underlying the StartReact effect by comparing onset latencies of voluntary ankle dorsiflexion and wrist flexion with and without a startling acoustic stimulus (SAS) in patients with hereditary spastic paraplegia (HSP) and age-matched healthy controls. Patients with HSP did have significantly delayed corticospinal motor conduction times to the leg muscles compared to reference values of healthy control subjects. In contrast, in patients with HSP, startle reflexes in the tibialis anterior (TA) muscle were not different from those in healthy controls with regard to both onset latencies and rates of occurrence. Simple reaction times of voluntary ankle dorsiflexion were delayed in the patients compared to the controls. However, when this task was combined with a SAS, reaction times in the patients were accelerated to a larger extent, resulting in completely normalized EMG and movement onset latencies. When the reaction time task involved voluntary wrist flexion instead of ankle dorsiflexion, we recorded no differences in onset latencies between patients and controls, irrespective of whether a SAS was applied. This pattern of results is consistent with the hypothesis that a SAS accelerates reaction times through a release of a subcortically stored motor program, conveyed by the reticulospinal tract.

Reticulospinal integrity in HSP

To test the function of the reticulospinal tract in patients with HSP, we used SAS to elicit startle reflexes in the SCM muscle, a muscle that is known to respond well to SAS. Furthermore, we recorded startle reflexes in the TA and flexor carpi radialis (FCR). The reticulospinal tract did not seem to be affected in the patients, as onset latencies and reflex occurrence in all three muscles were not different from controls. To our knowledge, this is the first study to test the function of the reticulospinal tract in patients with HSP. The finding that not all patients and control subjects expressed startle reflex activity can be considered as a limitation of this method of assessing reticulospinal tract function. However, to our knowledge, there is no alternative for in-vivo assessment of the functional integrity of the reticulospinal tract. Moreover, we found no indication that disease severity was anyhow related to the absence of TA startle reflexes in the patients.

Corticospinal degeneration in HSP

We were able to confirm the characteristic length-dependent, retrograde dysfunction of the corticospinal tract in HSP[311] using reaction time tasks involving both voluntary ankle dorsiflexion and wrist flexion. In the selected patients, ankle dorsiflexion was delayed and wrist flexion not, which is coherent with the prolonged corticospinal motor conduction times (CMCTs) to the lower limbs in patients with HSP. These prolonged CMCTs are in line with the literature as well as with the accepted notion of retrograde degeneration of the corticospinal tract.[38,177,253,281,289,312] In the patients, the delay in ankle dorsiflexion reaction times (19ms) was greater than the delay in CMCTs (6-7ms). As patients were stimulated at 100% of the stimulator output during the TMS procedure, the CMCTs likely reflect the conduction time in the least affected corticospinal axons. Reasonably, the conduction time is longer in axons that are more affected. During the reaction time task involving ankle dorsiflexion, it is unlikely that the response involved excitation of as many neurons and at the exact same time compared to the TMS-evoked response. Hence, it is conceivable that, with degeneration of the corticospinal tract, reaction times to a visual stimulus exhibit longer delays than those measured with TMS, because the (first) corticospinal neurons to depolarize are not the least affected.

The origin of the StartReact effect

In the present experiment, we accelerated reaction times using a SAS, a phenomenon known as StartReact.[359] Several observations strongly argue against the accelerated latencies simply being startle reflexes. First, during the ankle dorsiflexion task, the interval between TA en GM activation was not influenced by a SAS. This finding suggests that the SAS released the motor program without changing the characteristic agonist-antagonist activation pattern. If the SAS-accelerated latencies would have been due to a startle reflex, TA and GM would have been activated synchronously, resulting in a shorter interval between both muscles. Our observation of a constant agonist-antagonist interval during StartReact is also in line with the literature. Valls-Sole and colleagues (1999) investigated the effect of a SAS on two stereotyped EMG patterns: the triphasic agonist-antagonist-agonist burst pattern of wrist flexion and the rising on tiptoes from standing position. When a SAS was accompanied with the imperative signal, the onsets of these movements were significantly accelerated, while leaving the movement-specific EMG pattern fully intact. This observation was reproduced by Carlsen and colleagues.[54] A second argument against the SAS-accelerated latencies being startle reflexes is the observation that EMG activity in the TA was only observed during the ankle dorsiflexion task, and not during the wrist flexion task, and wrist flexor activity was observed only during the wrist flexion task and not during the ankle dorsiflexion task. This finding suggests that the SAS released a specific motor program and that the accelerated latencies were not the expression of startle reflexes.

Previously, three mechanisms have been proposed to explain the occurrence of the StartReact effect. One hypothesis states that a SAS may act as an additional stimulus on top of the imperative stimulus, thereby increasing the energy of the sensory input, resulting in an acceleration of sensorimotor coupling. This hypothesis, known as intersensory facilitation,[255] involves the corticospinal tract, both in trials with and without a SAS. Yet, the degeneration of the corticospinal tract in patients with HSP did not lead to an impaired StartReact effect in the present study. In line with previous studies that have refuted this hypothesis,[56,359] our results demonstrate that the StartReact effect cannot be explained by intersensory facilitation.

The currently dominant hypothesis states that the accelerated motor responses are due to the SAS directly releasing a subcortically stored pre-prepared motor program, which is then conveyed by the reticulospinal tract.[310,359] Yet, another hypothesis that has recently been proposed is that a SAS acts as a subcortically mediated trigger for a cortically stored motor program,[3,60] which mechanism would involve ascending reticular-cortical pathways and the corticospinal tract. This suggestion came from two studies using transcranial magnetic stimulation (TMS). Both studies showed a significant delay in the StartReact effect when TMS was applied over the motor cortex.[3,339] Although these results may support the involvement of cortical pathways in mediating the rapid release of a planned movement by a SAS, they do not rule out the possibility that the TMS-induced delay was due to reduced reticulospinal excitability through inhibitory effects of cortico-reticular projections. A recent study did indeed provide evidence that the reticular formation can be mediated by TMS.[124] Thus, we believe that our finding that patients with HSP did not show an impaired StartReact effect in the TA muscle strongly argues in favor of a SAS releasing a subcortically stored motor program that is conveyed by an intact reticulospinal tract. This notion does not imply that the cortex has no influence on the subcortical release of motor programs by a SAS, as subcortical motor preparation most likely involves cortical processing.[220]

Role of subcortical structures in motor preparation

Our results indicate that during motor preparation of voluntary ankle dorsiflexion and wrist flexion, motor programs become represented at subcortical levels which can be launched by a suitable reticular input. Indeed, studies in monkeys and cats provided evidence that motor preparation is not restricted to the cerebral hemispheres, and identified the pontomedullary reticular formation (pmRF) as one of the subcortical structures that subserves motor preparation as well.[45,315] As the pmRF is also a key structure in the startle reflex circuitry,[83] it may play a pivotal role in the release of pre-prepared motor programs, resulting in the StartReact effect.

The extent to which the reticular system is involved in motor preparation probably varies depending on the type of movement. It has been hypothesized that the reticular system is involved in grasping, but not in all tasks that require individuated finger movements.[163] Accordingly, the StartReact effect was absent in the first dorsal interosseous (FDI) muscle during index finger abduction, whereas a startle did accelerate FDI latencies during grasping.[163] It remains speculative why the reticular formation is differently involved in the preparation of various movements. As the reticular formation is a key structure in postural control,[337] the representation of intended movements at a reticular level might enhance their integration with anticipatory postural adjustments for the upcoming actions. Again, this does not imply that the motor cortex is not involved in anticipatory processes.[333]

The results from the present and previous StartReact studies provide strong evidence for the existence of potent reticulospinal control over coordinated movements of the hand and foot.[303] As such, the reticulospinal system may be responsible for some of the functional recovery observed after acute corticospinal lesions.[12] Recent experiments tested this idea by making focal unilateral pyramidal tract lesions in macaque monkeys.[387] After initial flaccid paralysis, grip function of the contralesional hand quickly recovered, which could not be attributed to corticospinal recovery. Interestingly, at six months post lesion, they demonstrated strengthening of reticulospinal connections to the forearm flexor, but not to the extensor muscle groups. This pattern mirrors the predominant recovery of upper extremity flexor function as observed in patients with corticospinal lesions such as stroke. The gain in reticulospinal output to forearm flexors in stroke patients is also supported by a recent study that showed normal StartReact responses in stroke patients during elbow flexion, whereas excessive flexor activity was seen in SAS-trials involving elbow extension. [162]

We suggest that in the case of HSP, there may indeed be some degree of neuro-plasticity through the reticulospinal system, thereby bypassing the dysfunctional corticospinal tract. Voluntary motor control through this bypass likely relies on intact cortico-reticular pathways that originate from the premotor cortex, descend through the corona radiata, and terminate at the pmRF.[385] This may explain why patients with HSP (in whom these pathways are likely unaffected) generally retain voluntary, but less refined control over their leg movements, much more so than people with a severely affected corticospinal and cortico-reticular tract after supratentorial stroke.

Conclusions and future perspectives

The results of this study in patients with pure hereditary spastic paraplegia support the hypothesis that the StartReact phenomenon can be attributed to the direct release of a subcortically stored pre-prepared motor program. Future studies may focus on the plasticity of the cortico-reticulospinal pathways in humans and their role

in motor control, as this may be a rather neglected substrate for functional recovery following lesions of the corticospinal tract.

6

Mechanisms of postural instability in hereditary spastic paraplegia

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Abstract

Hereditary spastic paraplegia (HSP) is characterized by progressive lower extremity spasticity and weakness, due to retrograde axonal degeneration of the corticospinal tract and posterior spinal columns. HSP patients fall frequently. We hypothesized that delayed postural responses contribute to their balance impairments. To distinguish between a delay in afferent and efferent signals, we combined postural responses with a startling acoustic stimulus (SAS). The SAS triggers a postural response directly, bypassing afferent proprioceptive input.

We performed two experiments. First, 18 HSP patients and nine healthy controls stood on a balance platform and were instructed to counteract forward and backward balance perturbations, without taking a step or grabbing a handrail. Second, 12 HSP patients and nine controls received backward perturbations, while a SAS accompanied onset of platform motion in 25% of trials.

HSP patients were less successful than controls in maintaining balance following backward and forward perturbations. Furthermore, latencies of postural responses were significantly delayed in HSP-patients, by 34 ms in gastrocnemius following forward, and by 38 ms in tibialis anterior following backward perturbations. A SAS accelerated postural responses in all participants, but more so in HSP patients whose latencies were normalized.

Our results suggest that delayed postural responses in HSP patients contribute to their balance problems. Combining balance perturbations with a SAS restored normal latencies, suggesting that conduction of efferent signals (presumably by the reticulospinal tract) is normal. We therefore suggest that the delayed postural responses in HSP are caused by slowed conduction time via the posterior spinal columns.

Introduction

Hereditary spastic paraplegia (HSP) is a heterogeneous group of disorders which are clinically characterized by progressive lower extremity spasticity and weakness.[150,311] The common pathological theme is retrograde axonal degeneration of the corticospinal tract, posterior spinal columns and, to a lesser extent, the spinocerebellar fibers.[149,228] Using transcranial magnetic stimulation, the retrograde axonal degeneration of the corticospinal tract has been demonstrated by the presence of prolonged central motor conduction time, elevated cortical motor thresholds and reduced amplitudes of motor evoked potentials of the legs.[38,177,253,281,289,312] Somatosensory evoked potential from lower limbs are frequently abnormal, demonstrating degeneration of the posterior spinal columns.[281,312] The reticulospinal tract is another long descending tract, but whether or not this tract is affected in HSP has not received any attention in the literature. Given its length, involvement in the retrograde degenerative process in HSP cannot be excluded.

A common problem in HSP are balance impairments, which result in falls. The balance impairments can only partly be explained by the lower extremity spasticity and weakness.[265] Therefore, we hypothesized that delayed onsets of postural responses may also contribute to balance impairments in HSP patients. Furthermore, we reasoned that delayed postural responses in HSP could be explained by either delayed conduction of afferent signals, by delays in efferent signals, or both. Afferent input following an externally imposed balance perturbation is conducted by the posterior spinal columns and integrated at the level of the brainstem and cortex.[176] The first efferent signals contributing to the postural response likely arise from the brainstem[176] and are conveyed by the reticulospinal tract.[161,290,315]

Here, we investigated the hypothesis that delayed postural responses contribute to balance impairment in HSP patients. Furthermore, we aimed to distinguish between a possible delay of signals in the afferent (posterior spinal columns) or efferent (reticulospinal) tracts, using balance perturbations both with and without a concurrent startling acoustic stimulus (SAS). A SAS can accelerate voluntary reaction times, a phenomenon known as 'StartReact'.[359] This StartReact effect can also be observed for postural responses that are elicited by sudden backward directed balance perturbations,[266] and might be explained by a direct release of a subcortically stored motor program, in this case the postural response, conducted by the reticulospinal tract.[359] As a result, afferent proprioceptive input becomes redundant for triggering a postural response when using a SAS. Therefore, if afferent signals are delayed in HSP, we expect to record normal onset latencies of muscle activity when the perturbation is combined with a SAS. In contrast, if efferent signals are delayed, we expect to record abnormal latencies during perturbations both with and without a SAS.

Materials and methods

We performed two separate experiments. In the first experiment, we examined postural responses to forward and backward perturbations. In the second experiment, we examined postural responses to backward balance perturbations and combined these with a SAS in 25% of trials.

Participants

Eighteen patients with autosomal dominant forms of HSP (AD-HSP) who were able to walk independently (12 men, mean 52 years, range 23-70) were recruited from a cohort HSP patients who visit or had visited the outpatient departments of our hospital. All 18 patients fulfilled the diagnostic clinical criteria for 'pure' HSP.[311] Eleven patients had previously been tested positive for pathogenic *SPAST* (SPG4) mutations, 1 patient for a pathogenic *KIAA0196* (SPG8) mutation, and 1 patient for a pathogenic *ATL1* (SPG3A) mutation. In the other five patients, mutations in genes most frequently associated with AD-HSP (*ATL1, SPAST* and/or *REEP1*) had been excluded. In addition, nine healthy controls in the same age range (7 men, mean 49 years, range 23-65) were included. All patients and controls participated in the first experiment. Twelve of the HSP patients (9 men, mean 51 year, range 23-70) and all the healthy control subjects participated in the second experiment. The study was approved by the local medical ethics committee and was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent prior to the experiment.

Clinical assessment

Muscle tone of the triceps surae and tibialis anterior muscles was assessed using the Modified Ashworth Scale (0-5), with higher scores indicating more hypertonia.[32] The triceps surae muscle was tested both with the knee flexed and extended. Muscle strength of the triceps surae and the tibialis anterior was assessed with the Medical Research Council (MRC) scale (0-5), with lower scores indicating less muscle strength.[76] We assessed the deep sensory modalities by testing the vibration sense at the lateral malleoleus and at the first metatarsophalangeal joint (MTP I) (0-8)(Rydel Seiffer, Neurologicals, Poulsbo, Washington), with lower scores indicating more sensory loss.[282] The mean value of vibration sense at the lateral malleolus and MTP I was taken. For all measures, the mean of both legs was determined and used for further analysis. Finally, the Berg Balance Scale (0-56) was used to assess sitting and standing balance, with lower scores indicating poorer balance control.[18]

Experimental setup and protocol

Participants stood on a moveable platform that could suddenly and unexpectedly translate in the forward and backward direction. A forward translation of the platform

resulted in a backward balance perturbation and vice versa; we will refer to the direction of the balance perturbation. Platform movements comprised an acceleration phase (300 ms), a constant velocity phase (500 ms) and a deceleration phase (300 ms). The magnitude of the balance perturbation was expressed in terms of acceleration. Participants were instructed to sustain the perturbations without taking a step or grabbing handrails, surrounding the platform, for support. Subjects wore a safety harness, which was attached to the ceiling, and prevented them from falling.

The first experiment involved forward and backward balance perturbations at three different levels of magnitude in each direction. Backward perturbations were delivered at 0.25m/s^2, 0.5m/s^2 and 0,75m/s^2 and forward perturbations at 0.5m/s^2, 0,75m/s^2 and 1.0 m/s^2. Each subject received four trials at each combination of direction and magnitude level (24 trials in total) in a random order. Consecutive trials were at least 20 seconds apart. Prior to the experiment, subjects received six practice trials (one for each acceleration level at both directions).

In the second experiment, subjects received 20 backward platform perturbations at 0,75m/s^2. In 25% of trials, the perturbation was combined with a SAS that was randomly administered through binaural earphones at the start of the platform movement. The SAS consisted of 50 ms of white noise with an intensity of 116 dB (SPL) and was generated using a custom-made noise generator. Again, subjects were instructed to sustain the perturbation without taking a step or grabbing the handrails for support.

Data collection

Muscle activity was measured using surface electromyography (EMG) data from bilateral rectus femoris, biceps femoris, tibialis anterior, and gastrocnemius medialis (ZeroWire by Aurion, Italy; 1000 Hz). Self-adhesive Ag-AgCl electrodes (Tyco Arbo ECG) were placed approximately 2 cm apart and longitudinally on the belly of each muscle, according to Seniam guidelines.[156] In addition, we recorded the platform movement and the signal of the SAS synchronously with the EMG data. In the first experiment, reflective markers were placed using a full-body model.[84] Marker positions were recorded by an 8-camera 3D-motion analysis system (Vicon Motion Systems, United Kingdom) at a sample rate of 100 Hz.

Data analysis

For each participant and for forward and backward directions separately, we calculated the percentage of trials in which subjects were successful in sustaining the perturbation without taking a step or grabbing the handrails.

EMG. EMG signals were full-wave rectified and low-pass filtered at 30 Hz (zero-lag, second order Butterworth filter). For each participant and for all combinations of perturbation magnitude and direction, the ensemble average EMG activity was calculated for each muscle. In the second experiment, this was done separately for trials with and without a SAS. Onset latencies of the various muscles were determined using a semi-automatic computer algorithm that selected the moment at which the ensemble average EMG activity exceeded a threshold of 2 SD above the mean background activity, as calculated over a 500 ms period just prior to perturbation onset. After its determination by the computer algorithm, onset latencies were visually checked and corrected as needed. For forward perturbations, we identified the onset latencies in the gastrocnemius and biceps femoris muscles. For backward perturbations, we determined the onset latencies in tibialis anterior and rectus femoris muscles. Latencies were clearly present in tibialis anterior and the medial gastrocnemius, but in the first experiment latencies could not be detected in rectus femoris in 7 participants and in biceps femoris in 8 participants, particularly during low magnitude perturbations. In one participant, a rectus femoris-latency could not be detected during the second experiment. For each muscle, onset latencies of the left and right leg were averaged, as there were no significant differences between the legs (neither when comparing the left and right leg ($p>0.05$), nor when comparing the leg with the greatest sensory loss to the contralateral leg ($p>0.05$)). The average EMG response amplitude was calculated over 100 ms following muscle onset, and corrected for background activity.

Motion analysis. For the first experiment, we determined the excursions of the extrapolated centre of mass (XCom) for each participant at each magnitude level and perturbation direction. The XCom integrates the position and velocity of the whole-body centre of mass.[159,160] For each trial we calculated the XCom excursion at 300 ms following the onset of the balance perturbation, because at this instant none of the participants had taken a step yet. The mean XCom displacement was calculated for each perturbation direction.

Statistical analysis

The number of successful trials was compared between HSP patients and controls using an independent samples t-test.

In the first experiment, onset latencies and amplitudes of muscle activity, were analyzed for each muscle separately, using repeated measures ANOVAs, with level of perturbation magnitude as within-subjects factor (*magnitude 1-magnitude 2-magnitude 3*) and group (*HSP patients- controls*) as between-subjects factor. The XCom displacement was analyzed in the same manner.

In the HSP group, we determined Pearson's correlation coefficients between the onset latencies of the prime mover of the postural response (tibialis anterior for backward perturbations and gastrocnemius for forward perturbations) and (i) the percentage of successful trials and (ii) the mean XCom excursion. In addition, a Spearman's rank correlation was performed between the onset latencies of tibialis anterior and gastrocnemius medialis and (iii) each of the clinical parameters.

In the second experiment, onset latencies and amplitudes of muscle activity, were compared between trials with and without a SAS using a repeated measures ANOVA, with SAS as within-subjects factor (*SAS -no* SAS) and group (*HSP patients-controls*) as between-subjects factor.

Results

Patients

The results of the clinical assessments are shown in Table 1. Lower limb spasticity was present in all patients, but was never severe (MAS≤2). Vibration sense was suboptimal or reduced in all patients, while lower limb muscle strength was relatively preserved (MRC always≥4).

Table 1 Clinical assessment scores of HSP patients (median (range)).

MAS tibialis anterior	0 (range 0-0)
MRC tibialis anterior	5 (range 4.25-5)
MAS triceps surae	2 (range 1-2) with knee extended; 1 (range 1-2) with knee flexed
MRC triceps surae	5 (range 4-5)
Vibration sense	4.5 (range 0.25 – 6.25)
BBS Score	54 (range 36-56)

MAS= Modified Ashworth Scale, MRC = Medical Research Council scale, BBS = Berg Balance Scale, Vibration sense tested with a semi-quantitative tuning fork (0-8).

First experiment

Success rate of balance recovery responses

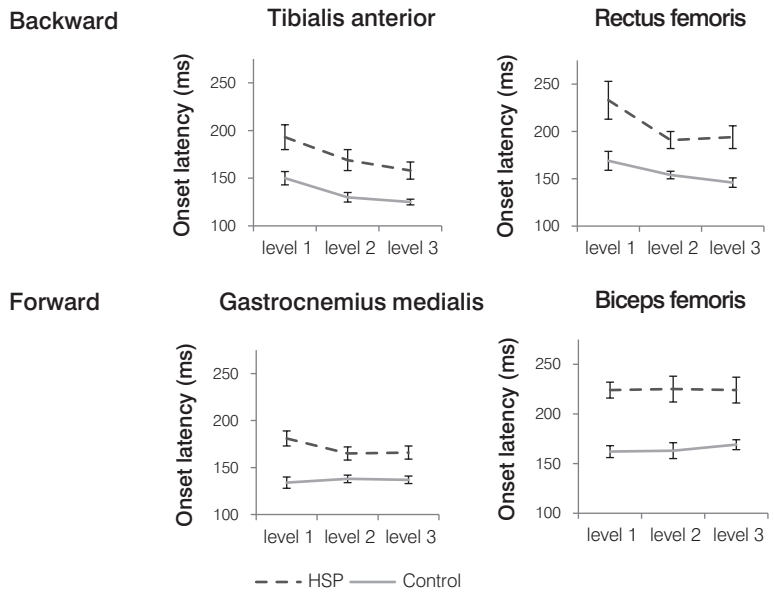
HSP patients were less successful compared to controls in maintaining balance without taking a step or grasping the handrails, in both forward (60±23% vs 89±14%, $p=0.002$) and backward perturbations (53±13% vs 82±18%, $p<0.001$). When HSP

patients were unable to sustain the perturbation with a feet-in-place strategy, some patients had a tendency to grasp for the rails, whereas healthy controls were inclined to make a step instead of grasping for the bars. A larger XCom displacement was seen in HSP patients compared to controls, during both forward (74±6 vs 66±14 mm/s, *group;* $F_{1,25}$=4.276, p=0.049) and backward perturbations (43±7 vs 34±10 mm/s, *group;* $F_{1,25}$=6.687, p=0.016). A higher perturbation magnitude resulted in a larger XCom displacement, during both forward and backward perturbations (*level;* $F_{1,25}$=534.642, p<0.001 and $F_{1,25}$=114.493, p<0.001, respectively), without a difference between both groups (*level x group*; $F_{1,25}$<0.253, p>0.777).

Onset latencies

Compared to controls, onset latencies following forward perturbations were delayed in HSP patients, by on average 34 ms in medial gastrocnemius (*group;* $F_{1,25}$=12.489, p=0.002) and 60 ms in biceps femoris (*group;* $F_{1,17}$=12.275, p=0.003). Onset latencies did not differ between perturbation magnitudes (*magnitude* and *magnitude x group* effects, all p values≥0.064; Figure 1).

Figure 1 Onset latencies (SE) of postural responses to backward (upper graphs) and forward perturbations (lower graphs). Perturbations were delivered at three levels of magnitude in each direction. Backward perturbations were delivered at 0.25m/s², 0.5m/s² and 0.75m/s² and forward perturbations at 0.5m/s², 0.75m/s² and 1.0 m/s².



Following backward perturbations, onset latencies in HSP patients were delayed by 38 ms in tibialis anterior (*group* $F_{1,23}$=6.640, p=0.017, Figure 1) and by 50 ms in rectus femoris (*group;* $F_{1,18}$=10.525, p=0.005, Figure 1). In contrast to the forward perturbations, onset latencies varied with perturbation magnitude. Onsets were significantly faster at larger accelerations (*magnitude;* tibialis anterior, $F_{2,22}$=25.585, p<0,001; rectus femoris, $F_{2,17}$=0.269, p=0.765), similarly for both groups (*magnitude x group*, p values ≥ 0.596).

Amplitude of postural responses

Following forward perturbations, the amplitudes of postural responses in gastrocnemius medialis were smaller in HSP patients compared to controls (*group;* $F_{1,25}$=6.265, p=0.019), whereas they were not significantly different in biceps femoris (*group;* $F_{1,17}$=0.996, p=0.332; see Table 2). In both groups, response amplitudes increased with higher perturbation magnitudes, both in gastrocnemius (*magnitude;* $F_{2,24}$=5.547, p=0.010) and in biceps femoris (*magnitude;* $F_{2,16}$=13.400, p<0.001).

Following backward perturbations, rectus femoris amplitudes were decreased in HSP patients (*group;* $F_{1,17}$=4.831, p=0.041; see Table 2), whereas tibialis anterior amplitudes were not significantly smaller (*group;* $F_{1,23}$=2.361, p=0.138). Similar to the forward perturbations, response amplitudes increased with higher perturbation magnitudes, both in tibialis anterior (*magnitude;* $F_{2,22}$=22.039, p<0.001) and rectus femoris (*magnitude;* $F_{2,16}$=43.539, p<0.001).

Table 2 Mean (SD) EMG amplitudes first experiment.

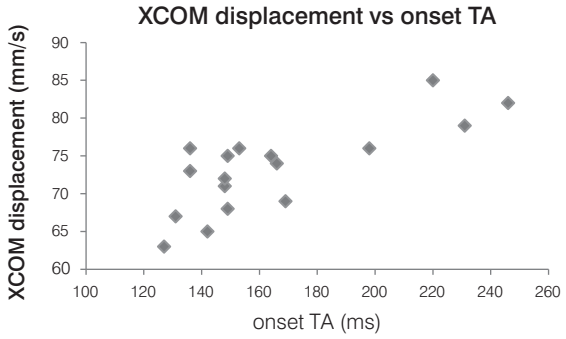
	Perturbation direction	HSP	Control
Gastrocnemius medialis	Forward	0.040 (0.026) mV	0.071 (0.040) mV
Biceps femoris	Forward	0.019 (0.010) mV	0.023 (0.017) mV
Tibialis anterior	Backward	0.115 (0.080) mV	0.161 (0.073) mV
Rectus femoris	Backward	0.022 (0.020) mV	0.037 (0.021) mV

Correlations between onset latencies and balance recovery

In HSP patients, longer onset latencies moderately correlated with larger XCom excursions at 300 ms following the start of the perturbation (r_p=0.669; p=0.002, Figure 2) and strongly with reduced success in sustaining the forward perturbation without stepping or grabbing for support (r_p=-0.752; p<0.001).

Delayed onset latencies in tibialis anterior correlated strongly with further backward XCom excursions (r_p=0.760; p<0.001, Figure 2), but not with balance recovery success rates (r_p=-0.317; p=0.199).

Figure 2 The relation between onset latencies of(prime movers of) the postural response and XCom displacements in HSP patients. TA=tibialis anterior, GM=gastrocnemius medialis.



Correlations between onset latencies and clinical assessments

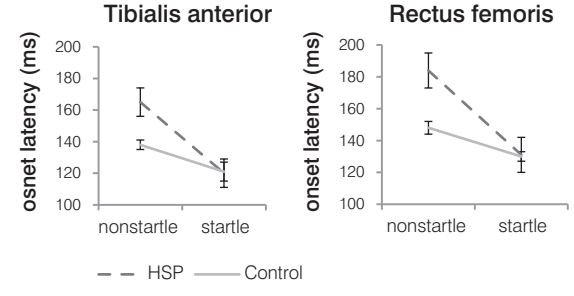
Longer onset latencies in gastrocnemius moderately correlated with decreased vibration sense (r_s = -0.582; p=0.014) and strongly with triceps surae muscle strength (r_s=-0.718; p=0,001), but not with muscle tone (r_s=-0.315; p=0.235). The triceps surae muscle strength moderately correlated with XCom excursions (r_s= -0.675; p=0.003) and strongly with reduced success in sustaining the forward perturbations (r_s=0.724; p=0.001). Larger onset latencies in gastrocnemius strongly correlated with triceps surae muscle strength (r_s=-0.718; p=0.001).

Longer onset latencies in tibialis anterior also correlated moderately with decreased vibration sense (r_s=-0,625; p=0.007), but not with muscle strength in tibialis anterior (r_s=0.227; p=0.364). The muscle strength in tibialis anterior did not correlate with XCom excursions nor with successfulness of sustaining backward perturbations (r_s=0.324 ; p=0.190; r_s=-0.269 and p=0.281, respectively).
Furthermore, higher scores on the Berg Balance Scale moderately correlated with longer onset latencies in tibialis anterior and gastrocnemius (r_s=-0.492 ; p=0.045; r_s=-0.467 and p=0.059, respectively).

Second experiment

Both in HSP patients and controls, the SAS accelerated the postural responses in tibialis anterior and rectus femoris (*SAS;* $F_{1,19}$=49.203, p<0.001 and $F_{1,18}$=43.042, p<0.001, respectively). The HSP group, however, benefited from the startle to a much larger extent compared to the controls, with a 45 ms reduction in tibialis onset latencies and 53 ms in rectus femoris, versus 17 ms and 18 ms in the controls (*SAS x group;* $F_{1,19}$=10.330, p=0.005 and $F_{1,18}$=10.069, p=0.005, respectively; Figure 3). In fact, the onset latencies with SAS in the HSP patients were no longer significantly different from those in the controls.

Figure 3 Onset latencies (SE) of postural responses during backward perturbations, with and without a SAS.



Response amplitudes in tibialis anterior were smaller in HSP patients compared to controls (*group;* $F_{1,19}$=4.587, p=0.045, see Table 3), whereas the response amplitudes in rectus femoris did not differ significantly (*group*; $F_{1,18}$=1.441, p=0.247). The SAS decreased the responses amplitudes in tibialis anterior and tended to reduce the amplitudes in rectus femoris (*SAS;* $F_{1,19}$=15.415, p=0.001 and $F_{1,18}$=4.227, p=0.056, respectively). The effect of the SAS on the response amplitudes did not differ between the two groups (*SAS x group;* $F_{1,19}$=0.001, p=0.977 for tibialis anterior and $F_{1,18}$=0.795, p=0.386 for rectus femoris).

Table 3 Mean (SD) EMG amplitudes second experiment.

	HSP		Control	
	Nonstartle	Startle	Nonstartle	Startle
Tibialis anterior	0.112 (0.060) mV	0.090 (0.056) mV	0.165 (0.055) mV	0.143 (0.054) mV
Rectus femoris	0.024 (0.016) mV	0.018 (0.010) mV	0.031 (0.012) mV	0.028 (0.012) mV

Discussion

This study investigated the hypothesis that delayed postural responses contribute to balance impairment in HSP patients. Furthermore, we aimed to distinguish between a possible delay of signals in afferent versus efferent tracts, using balance perturbations both with and without a concurrent startling acoustic stimulus (SAS). HSP patients were indeed more unstable compared to controls, as reflected by the larger percentage of trials in which they had to take a corrective step or grasp the handrails. This greater instability was seen following both forward and backward balance perturbations. Furthermore, postural responses were delayed in HSP patients following balance perturbations in either direction. When backward balance perturbations were accompanied by a SAS, the onset latencies of muscle activity in the prime movers significantly accelerated. This acceleration of postural responses was larger in HSP patients compared to healthy controls, resulting in normalized latencies for these patients.

Delayed postural responses are associated with postural instability in HSP

The reduced ability of HSP patients to successfully resist balance perturbations recalls previous work by our group.[86] Here, we demonstrate that delayed postural responses are associated with reduced limits of stability in HSP patients, in terms of their ability to successfully maintain balance at least in the forward direction. In addition, the severity of the delay in muscle activity correlated with a greater displacement of the XCom (extrapolated centre of mass) in both the backward and forward directions. The delay in muscle activity correlated with the successfulness of maintaining balance after forward, but not after backward perturbations. Some HSP patients experienced the backward perturbations as more frightening than the forward perturbations. Particularly for low acceleration trials, they sometimes seemed to be able to successfully recover their balance, but at the very last moment they made a small step or grasped for support to ascertain their recovery. This might explain why the degree of delay in muscle activity did not significantly relate to the successfulness of maintaining balance after backward perturbations, but still correlated with a greater backward displacement of the XCom. The greater the displacement of the XCom, the more balance is threatened. Hence, our results suggest that delayed postural responses contribute to balance impairments in HSP patients in both the forward and backward direction.

Delayed postural responses are caused by impaired afferent input

To distinguish between the afferent and effect contribution to the postural responses, we accelerated these responses using a startling acoustic stimulus (SAS). By combining

backward balance perturbations with a SAS, afferent input becomes redundant for triggering the postural response.[266] When backward perturbations were combined with a SAS, the latencies normalized in the HSP patients, which suggests there was no delay in the conduction of the efferent signals conveyed by the reticulospinal tract. Hence, we suggest that the observed delay in the postural responses was caused by slowed conduction of afferent signals in the posterior spinal columns of HSP patients. Likely, all postural responses which rely on afferent information conducted by the posterior spinal columns will be delayed in HSP patients. The suggestion of prolonged conduction time in the posterior spinal columns is in line with previous findings using somatosensory evoked potentials.[99,281] The inference between prolonged afferent conduction time and delayed postural responses was also supported by the observed associations between reduced vibration sensory and delayed postural responses, and is in agreement with a previous study that reported that in patients with multiple sclerosis, slowed spinal somatosensory conduction was associated with delayed postural responses.[48]

In contrast, the present findings are not in line with a previous study reporting intact latencies of short and medium latency postural responses in 4 HSP-patients.[251] This discrepancy may be explained by the fact that the latter study included patients without sensory impairment. In addition, we used translational perturbations, whereas the study of Nardone *et al.* used rotational perturbations. It cannot be ruled out that the afferent input of rotational perturbations is (partly) conveyed by different yet intact sensory spinal pathways, and this requires further study.

Comparison of response amplitudes between groups

In addition to the delayed onset latencies, HSP patients also demonstrated smaller amplitudes of their postural responses (17-44% reduction), although not significantly demonstrated for all the muscles. This could be explained by our relatively small sample size and by the relatively large variability between participants. Despite the obvious limitations inherent to the comparison of EMG amplitudes between subjects, the decreased amplitudes could indicate that muscle weakness also contributes to the balance impairments, which is in line with the observation that decreased strength in the triceps surae muscles correlates with successful balance maintenance following perturbations.[86] A further mechanism that may contribute to the decreased amplitudes in HSP patients could be a diminished corticospinal drive onto spinal interneurons. Postural responses are generated within reticular structures and are conducted by the reticulospinal tract, but the ultimate expression of postural response is determined by the excitability of interneurons within the spinal cord.[290] The excitability of these interneurons depends on corticospinal drive,[316] which is likely reduced in HSP patients due to axonal degeneration of this tract. Our observation

that the SAS normalized the latencies, but without normalizing the response amplitudes, suggests that the decreased response amplitudes may indeed be due to this mechanism.

Limitations

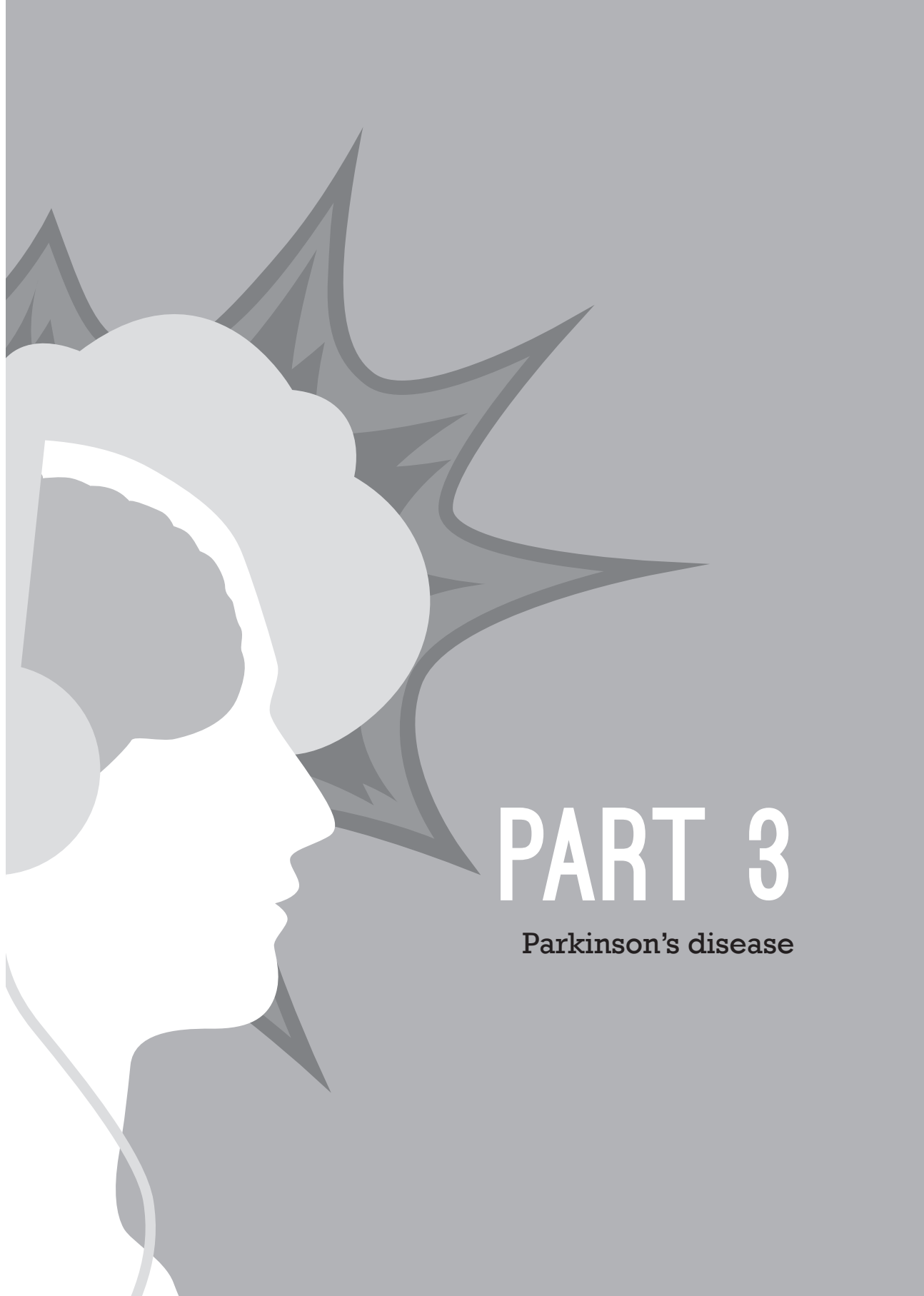
Previous work showed that the acceleration of movement latencies by a SAS can have functional benefits.[293] Here, we did not measure whether the accelerated postural responses by a SAS had beneficial effects. This would have further contributed to the notion that delayed postural responses contribute to balance impairments. Future studies should focus on further substantiation of the notion that delayed postural responses are a cause of balance impairments and falls in HSP.

Implications

Our study raises the question whether sensory feedback training could reduce postural instability in patients with HSP. Artificial biofeedback mechanisms are able to supplement natural sensory inputs, by providing additional sensory information to the brain by means of auditory, vibrotactile or visual feedback. Several studies have already shown that direct biofeedback can improve postural stability in healthy young [171] and elderly subjects.[366] Furthermore, similar benefits have been shown in vestibular loss patients.[168,372] Sensory feedback can also be implemented in a training program, with promising results reported both in healthy subjects[82] and in patients with Parkinson's disease.[250] It remains for future research whether sensory feedback training may also be helpful in patients with HSP.

Conclusion

Balance impairments and consequent falls are a significant problem in patients with HSP. Our results suggest that delayed postural responses contribute to the balance impairments in HSP patients, in addition to loss of muscle strength. The delayed postural responses are likely due to a longer conduction time in posterior spinal columns, whereas the efferent conduction time in the reticulospinal tract seems to be unaffected.



PART 3

Parkinson's disease

7

Unraveling the mechanisms underlying postural instability in Parkinson's disease using dynamic posturography

Published as:
Nonnekes J, de Kam D, Geurts AC, Weerdesteyn V, Bloem BR. Unraveling the mechanisms underlying postural instability in Parkinson's disease using dynamic posturography. Expert Review of Neurotherapeutics 2013;13:1303-8.

Abstract

Postural instability, one of the cardinal symptoms of Parkinson's disease (PD), has devastating consequences for affected patients. Better strategies to prevent falls are needed, but this calls for an improved understanding of the complex mechanisms underlying postural instability. We must also improve our ability to timely identify patients at risk of falling. Dynamic posturography is a promising avenue to achieving these goals. The latest moveable platforms can deliver 'real-life' balance perturbations, permitting study of everyday fall circumstances. Dynamic posturography studies have shown that PD patients have fundamental problems in scaling their postural responses in accordance with the need of the actual balance task at hand. On-going studies evaluate the predictive ability of impaired posturography performance for daily life falls. We also review recent work aimed at exploring balance correcting steps in PD, and the presumed interaction between startle pathways and postural responses.

Introduction

Parkinson's disease (PD) is a common neurodegenerative disease, with postural instability as one of the cardinal features of advanced disease stages.[25,72,288] Postural instability may result in falls that can have a devastating impact, due to both injuries and fear of falling and, subsequently, loss of mobility, physical and psychosocial decline and a reduced quality of life. In a prospective twenty-year follow-up of 136 patients with newly diagnosed PD, it was shown that the prevalence of falls is as high as 87%, with a fracture rate of 35%.[154] Although there is no conclusive evidence yet, it has been suggested that survival is reduced once falls have occurred.[225,375] Unfortunately, currently available medication has no or little effect on postural instability in PD.[23,62,191,192] Therefore, better management strategies to prevent falls are urgently needed. Developing such strategies requires the ability to identify patients who are at risk of falling and, therefore, also requires an improved understanding of the complex mechanisms underlying postural instability. In this narrative review, we describe how dynamic posturography can help to achieve these goals. Importantly, we do not intend to provide a comprehensive listing of all available publications on postural instability in PD, but rather report on some interesting recent developments within the field of dynamic posturography, aiming to illustrate the possible merits of this promising technique. We highlight how state-of-the-art dynamic posturography techniques could help to timely identify patients who are at risk of falling. Furthermore, we discuss how these innovations may contribute to an improved understanding of the pathophysiology of postural instability in PD, as a necessary basis for improved treatments and preventive strategies.

Dynamic posturography

Posturography investigates the regulation of balance under different conditions, and can be divided into static and dynamic techniques. During static posturography, postural control is assessed while participants maintain stance in an unperturbed state. Dynamic posturography, on the other hand, is an umbrella term for techniques that employ physical perturbations of stance.[28] The ecological validity seems to be higher for dynamic posturography compared to static posturography, as falls in daily life seldom result from static conditions, but rather from balance perturbations such as stumbling over an obstacle. In this narrative review, we will focus on dynamic posturography. One method to evoke balance perturbations is by using motorized platforms, of which movement of the support surface results in balance perturbations that are standardized across different subjects. Another method used in dynamic posturography is the waist pull paradigm, in which a standardized force is suddenly applied to the waist.[231] A third example is tendon vibration, where mechanical vibration of the tendon or muscles induces illusory sensations of movement.[364] In the case of

Achilles tendon stimulation, calf muscles will be activated and the body will move backwards.

Postural reactions can be quantified by analyzing ground reaction forces, muscle activation patterns (electromyography), and kinematic variables (analysis how body segments move). A measure for postural instability could be the number of compensatory steps that are needed to recover from a balance perturbation or the mechanical efficiency of the first balance corrective step expressed as body configuration at foot contact.[373]

Conventional dynamic posturography techniques, for instance using motorized platforms such as the Neurocom platforms,[67] have already produced valuable insight into the physiology of postural control in man and the pathophysiology of postural control in PD. For example, the predominant instability in backward direction has been confirmed by several studies that used rapidly moving (translating or rotating) platforms.[62,167] Still, it has not been unravelled lesions in which neural structures primarily underlie postural instability in PD. Furthermore, it remains difficult to predict individual fall risk. This is partly explained by the poor ecological validity of the conventional posturography equipment.

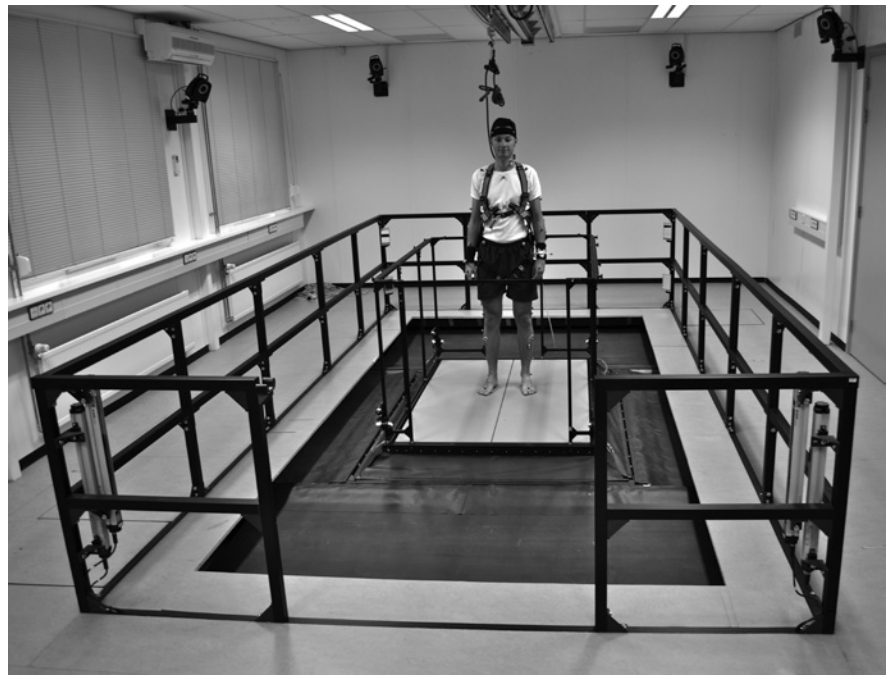
A first limitation of the commonly used motorized platforms, is the relatively small trajectory over which the platform moves.[369] This implies that the stimulus profile often consists of an initial quick acceleration, which is directly followed by a deceleration when the end of the platform movement is reached. The subsequent deceleration force has a significant impact on postural stability, because it helps subjects to recover from the balance perturbation. Furthermore, the muscle responses that are elicited by both the acceleration and deceleration pulse may become blended.[64,229] These problems can be overcome by the delivery of perturbations with large movement trajectories, which enable the postponement of the deceleration phase.

A second drawback is that not all balance platforms are large enough to allow participants to take corrective steps, as they would do in daily life when a fall is imminent. A final drawback of some conventional experimental setups is that even the highest perturbation intensities are insufficiently destabilizing to actually bring mildly affected PD-patients, without clinically evident postural instability, near or beyond their stability limits. These patients may, however, have subtle balance impairments.[230] When the perturbation intensity is too low, these impairments could remain undetected. Manipulation of somatosensory or visual input can be used to destabilize mildly affected patients, but this has a limited ecological validity as patients can rely on those systems in daily life. Recent innovative designs have overcome the described limitations, an example of which is demonstrated in Box 1.

Box 1 Radboud Falls Simulator

The recently developed Radboud Falls Simulator (see Figure1) is an example of a recently developed platform that allows studying postural responses under 'real-life' fall circumstances. The participant stands on two force plates (120x180cm, extendable to 174x240cm) and wears a safety harness that is attached to the ceiling. The suspension on the ceiling moves along with the platform. The Radboud Falls Simulator is able to deliver balance perturbations with very large amplitudes (translations up to 3 meters, maximum acceleration 4.5 m/s^2) in multiple (random) directions. In this way subjects are not able to preselect direction-specific responses. Translations as well as rotations (up to 9^o) can be delivered, both in isolation and coupled. The employment of different perturbation types provides complementary insights into the spectrum of balance responses. The Radboud Falls Simulator is able to flexibly postpone the deceleration phase of the platform movement, so that the platform does not create stabilizing forces. As such, highly destabilizing perturbations can be delivered, permitting analysis of critical fall restorative mechanisms around and past the limits of stability. The support surface consists of two very large, custom-made force plates, which jointly create a wide support surface that allows for multiple corrective steps in the anterior-posterior direction, and at least one large corrective step sideways.

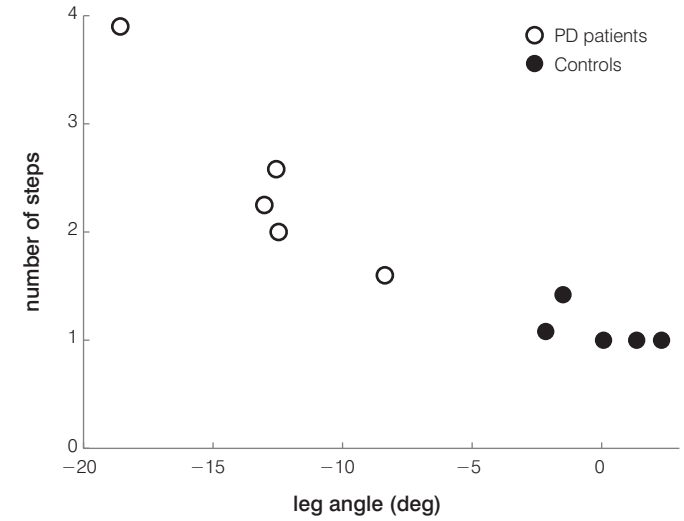
Figure 1 Radboud Falls Simulator.



Identification of fallers

Currently, there is much interest in fall prevention programs for PD patients, and new programs are being developed,[243] although few existing programs have actually succeeded to reduce the risk of falling.[205] It is, however, neither feasible nor necessary to offer each PD patient fall preventive treatment. Ideally, only those patients with an increased fall risk should be referred to such a fall prevention program. Hence, there is a need to identify those patients who are prone to falling. In this paragraph we discuss how dynamic posturography may add to the currently available methods to identify people at risk of falling. So far, clinical balance and gait performance tests have been used to determine individual fall risk. A drawback of most clinical tests is the inherent difficulty in standardizing performance, and the subjective scoring of the outcome. Many single clinical balance tests, such as the Berg Balance Scale (BBS), appear to have a poor predictive value with respect to falls.[17,92] Recently, the Mini BEST test[105] was demonstrated to have a higher sensitivity and specificity than the BBS in predicting falls in PD patients, yet a fall history remains a stronger predictor.[105] Furthermore, predicting the very first fall remains difficult using clinical balance tests.[105,285] From a clinical perspective, however, it would be preferable to identify people at risk before their very first fall has occurred. This calls for the identification of new, sensitive measures of postural instability that mark the transition from non-faller to faller status. Objective measures as spontaneous postural sway have previously been proposed, but failed to reliably distinguish fallers from non-fallers.[369] Recent findings provide arguments for the notion that dynamic posturography may be suitable to predict the transition from non-faller to faller. First, dynamic posturography is capable of revealing postural impairments in PD patients without clinically detectable instability,[230] indicating that this method is more sensitive than clinical testing. Second, recent developments within the field of dynamic posturography allow the detailed study of compensatory stepping, which ability is essential to prevent a loss of balance becoming a fall. It has already been shown that impaired compensatory stepping is associated with higher risk of falling in healthy older people and in people after stroke.[157,218] We hypothesize that impaired compensatory stepping is an important determinant of fall risk in PD patients as well, as compensatory stepping is often impaired in patients with PD.[191,192] The quality of backward compensatory stepping can be evaluated by looking at the mechanical efficiency of the step in terms of leg inclination angle at foot contact.[373] Preliminary results obtained with the Radboud Falls Simulator suggest that PD patients have a poorer mechanical efficiency of backward balance corrective steps compared to controls. This seems to be associated with a larger number of steps needed to recover from the perturbation (see Figure 2). Currently we are investigating whether the mechanical efficiency of the first balance correcting step could help to identify patients who are prone to fall. Concluding, dynamic posturography certainly holds promise in identifying those PD patients at risk of becoming a faller.

Figure 2 Quality of backward balance corrective steps in Parkinson's Disease. Mechanical efficiency of the first balance corrective step was quantified as the leg inclination angle at foot contact. Positive leg angles represent higher mechanical efficiency. Data represent the individually averaged values of 16 trials of backward perturbations on the Radboud Falls Simulator at a fixed level of intensity (1.5 m/s^2).



Underlying mechanisms

Although experienced clinicians are well capable of identifying patients with postural instability, these observations do not allow differentiating 'primary' pathology from 'secondary' compensatory strategies.[369] For the development of fall-preventive interventions, however, it is essential to distinguish between those phenomena, since those interventions should aim to restore primary impairments and facilitate effective, but discourage maladaptive compensatory strategies. With dynamic posturography it is possible to separate the different components of balance recovery responses. When balance is perturbed, afferent proprioceptive input triggers an automatic postural response (APR) from brainstem reticular structures.[161,176] Shortly after the start of the APR, transcortical pathways become involved, and these are particularly important when making a step or when grasping for support.[176] Most research on postural responses in PD has been done on the APRs.

A key finding from studies on APRs is that PD patients have fundamental problems in the scaling of their responses,[23,62,100,336] whereas the latencies of the postural responses appear to be normal.[252] The abnormal scaling of postural responses becomes even more apparent when the postural set is manipulated. The term

postural set covers a wide array of conditions. The manipulations include, for example, changes in the subject's initial body position and the subject's perceptions of the upcoming balance perturbation.[368] For example, when young healthy subjects receive a random mixture of small and large perturbations, they select a default postural response that is sufficiently large to cope with the largest possible perturbation.[15] In contrast, PD patients consistently exhibit response amplitudes that match the small perturbations, even when it is known in advance that a large perturbation will follow.[16] Although APRs may contribute to recovering from small balance perturbations, recovering from larger perturbations critically relies on stepping responses, as they represent a final common saving strategy to prevent falling. In line with the aforementioned studies on automatic postural responses in PD, the balance correcting steps seem to be under scaled in PD as well.[175,191,192] In PD patients, abnormal central proprioceptive-motor integration (rather than deficiencies in the afferent proprioceptive information itself) likely plays a role in the abnormal scaling of the balance responses.[31,175,189] Visual information is likely able to compensate for the abnormal proprioceptive-motor integration.[182,363] This could explain why patients with PD are most unstable to backward perturbations,[62,167] where compensation using visual input is not possible. Degeneration of dopaminergic circuits within the basal ganglia is thought not to be primarily responsible for the abnormal proprioceptive-motor integration, as medication does not consistently improve the balance responses.[23,62,175] However, the marginal effect of dopaminergic medication does not necessarily preclude a role for dopamine deficiency in the underlying pathophysiology, because the threshold for therapeutic improvement may be higher than for other symptoms.[142] In addition, postural instability in PD is typically seen in a relatively progressed disease stage, when adverse effects of dopaminergic medication preclude prescription of doses sufficiently high to improve postural performance. Finally, due to further spread of disease pathology, non-dopaminergic brain lesions develop which increasingly dominate the clinical presentation, masking the relative importance of the dopa-sensitive symptoms.

Future studies should further investigate deficient neural substrates underlying postural instability in PD. For example, studies conducted in patients with deep brain stimulation can give more insight in the contribution of different neuro-anatomical substrates to postural instability in PD.[336]

First trial responses

Recent studies have emphasized the unique nature of responses to the very first balance perturbation, the so-called first trial reactions.[5] This first and unpractised trial is typically excluded from further analyses, because the response is different from the reaction elicited during the following trials.[279] However, the analysis of the first trial responses could provide valuable information, as falls in daily life result from

unexpected and single events. The first studies evaluating first trial responses in PD have been conflicting, as one study reported no differences in first trial responses between PD patients and controls,[370] whereas a recent study reported significantly greater instability during first trials responses in PD patients.[249] Moreover, in the latter study, patients had a slower habituation rate across trials. One fascinating but still unanswered question is whether analysis of first trial responses offer better predictive value for falls in daily life, as compared to the averaged response to a series of identical balance perturbations (as was traditionally done until now).

Interestingly, there are several observations that suggest that first-trial responses partially consist of startle-like responses.[249,279,280] First, during the first trial responses, exaggerated trunk flexion is seen, which is comparable with the startle reflex following a startling stimulus. Secondly, startle reflexes habituate rapidly, just like postural responses.[41] Furthermore, two studies have reported absent or reduced startle reflexes in PD patients with postural instability.[349,367] The possible interaction between startles and postural responses can be investigated using the StartReact paradigm. In this paradigm, a startling acoustic stimulus (SAS) is given simultaneously with an imperative signal to initiate a movement, resulting in substantial acceleration of movement onsets.[359] Our recent work has demonstrated that this phenomenon also applies to APRs to backward perturbations, when a SAS is delivered at the same time as the balance perturbation.[266] Interestingly, a recent study showed that patients with both severe freezing of gait and postural instability had an absent StartReact effect when performing a simple ballistic movement, which was restored by stimulation of the pedunculopontine nucleus (PPN).[349] As such, applying the StartReact paradigm to postural perturbations could provide further insight into the possible interactions between postural responses and startle pathways, thereby giving further clues with regard to targets for deep brain stimulation to alleviate postural instability.

Expert commentary

Postural instability and falls are debilitating features of PD. The underlying mechanisms remain poorly understood, hampering targeted therapeutic management. Recent developments in dynamic posturography hold promise to expand our knowledge on this important topic, with the latest balance platforms permitting study of every day fall circumstances, such as mimicked slips or unpredictable falls in different directions. Furthermore, analysis of first trial responses and stepping to recover balance at the very limits of stability will further enhance the ecological validity of findings from these studies.

Dynamic posturography is not yet ready to be applied in current clinical practice to identify individual patients who are at risk of falling, as measures of postural instability

that mark the transition from non-faller to faller status are currently lacking. An improved understanding of deficient neural substrates underlying postural instability in PD will not only facilitate the search for such a marker, but will also open up new windows for pharmacological, neurosurgical, or training interventions.

Five year view

In five years time, the underlying mechanisms of postural instability in PD will be further unravelled. We will know to what extent postural responses are related to startle pathways. Furthermore, the underscaling of balance correcting steps in PD will be investigated in more detail. We expect that if the pathophysiology of postural instability in PD is further unravelled, we can start with the identification of patients who are prone to falling before they actually start to fall. In that stage, it is our expectation that dynamic posturography can be applied in the clinical diagnostic management of individual patients.

Key issue

- Postural instability is a frequent and debilitating, yet poorly understood symptom of Parkinson's disease (PD).
- PD patients have a fundamental problem in scaling their postural responses, partly due to abnormal proprioceptive-motor integration.
- Dynamic posturography is a potential tool to unravel the mechanisms underlying postural instability in PD.
- Recent innovations in posturography allow for delivering 'real-life' balance perturbations, permitting study of everyday fall circumstances.
- Dynamic posturography cannot yet be clinically applied to identify PD patients prone to falling, but more work remains needed in this area.
- Future research in patients with PD should study the balance correcting steps at the limits of stability and the possible interaction between startles and postural responses.

8

Reduced StartReact effect and freezing of gait in Parkinson's disease: two of a kind?

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Abstract

Freezing of gait (FOG) is a disabling feature of Parkinson's disease. Emerging evidence suggests that dysfunction of the PPN and pmRF play a role in the causation of FOG. These brainstem structures can be examined by the StartReact paradigm, which utilizes a startling stimulus to accelerate reaction times (StartReact). Here, we examined gait initiation in PD-patients with and without FOG using this paradigm.

Twenty-six patients with Parkinson's disease (12 freezers and 14 non-freezers) and 15 controls performed two tasks: rapid gait initiation in response to an imperative 'go' signal; and a control condition, involving a simple reaction time task involving ankle dorsiflexion. During both tasks a startling acoustic stimulus was combined with the imperative signal in 25% of trials.

In controls, the startle accelerated gait initiation and shortened the onset latency of tibialis anterior responses during ankle dorsiflexion. This acceleration was intact in non-freezers, but was significantly attenuated in the freezers. Independent of the occurrence of a startle, freezers showed a reduced length of the first step compared to non-freezers and controls.

The diminished StartReact effect in freezers probably reflects deficient representation or release of motor programs at brainstem reticular level due to dysfunction of the PPN, the pmRF, or both. These brainstem structures are presumably involved in integrating anticipatory postural adjustments with subsequent stepping movements. We suggest that with time-varying demands, these structures may no longer be able to coordinate the integration of anticipatory postural adjustments with steps, leading to FOG-episodes.

Introduction

Freezing of gait (FOG) is a disabling feature of Parkinson's disease (PD).[25] The underlying pathophysiology is still poorly understood. There is emerging evidence that dysfunction of the pedunculopontine nucleus (PPN) and pontomedullary reticular formation (pmRF) play a role in causing FOG.[275] Dysfunction of these brainstem circuits in PD-patients with FOG has recently been suggested by a study that evaluated the so-called 'StartReact' paradigm.[349] In the StartReact paradigm, a startling auditory stimulus (SAS) accelerates the latencies of movement responses to an imperative 'go' signal. The accelerated movement onsets during StartReact experiments are dissociated from startle reflexes,[349] and are thought to reflect a direct subcortical release of motor programs from the pmRF.[55,271,359] The StartReact effect was absent in PD-patients with severe FOG performing a simple ballistic movement of the upper extremity, but was intact in non-freezers.[349] Remarkably, PPN-stimulation restored the SAS-induced movement onset acceleration.[349] Although restoration of this StartReact effect seemed to be associated with perceived improvements in gait,[350] the question remains whether and how deficient StartReact effects of the upper extremity may relate to FOG. We reasoned that demonstration of an impaired StartReact effect in a gait-related task would provide stronger support for the relevance of upper brainstem dysfunction in FOG. We therefore examined gait initiation in freezers and non-freezers using the StartReact paradigm. We added an ankle dorsiflexion task as a control condition, aiming to reproduce the StartReact effect for a simple ballistic movement.[349] We predicted that the StartReact effect would be absent or reduced in freezers during gait initiation as well as ankle dorsiflexion.

Materials and methods

Participants

Twenty-six patients with PD participated: 12 with FOG and 14 without FOG (see below for definitions). Exclusion criteria were any other disorder or medication affecting gait and severe cognitive impairment. Patients were measured in an OFF-state, when they experienced an end-of-dose effect prior to intake of their next medication dose. In addition, 15 healthy controls of similar age were included. The study was approved by the local medical ethics committee. All subjects gave their written informed consent prior to the experiment.

Clinical assessment

PD-patients were clinically assessed with the motor subsection (Part III) of the MDS-Unified Parkinson's Disease Rating Scale (UPDRS, score/132).[137] Patients also completed

the New Freezing of Gait Questionnaire (N-FOGQ, score/33).[258] Additionally, they performed a series of walking tests to objectively verify subjects as freezers or non-freezers,[331,332] including eight rapid axial 360-degree turns in both directions and walking with 25% of the preferred step length (at a normal pace, and as rapidly as possible). Based on the detailed physical examination, 12 persons were classified as 'freezers', and the 14 others were classified as 'non-freezers' as they did not show FOG-episodes during examination, and never experienced subjective gluing in daily life. The N-FOGQ revealed that all freezers had more frequent and more severe FOG during the OFF-medication state. Additionally, global executive function was assessed with the Frontal Assessment Battery (FAB, score/18).

Experimental setup and protocol

First, participants performed a warned reaction task. For this test, participants sat in a chair placed in front of two blocks with light-emitting diodes (LEDs). Illumination of the first LED-array served as a warning signal and participants were instructed to perform ankle dorsiflexion as soon as the second LED-array was lit. The latter was the imperative stimulus (IS). Patients performed ankle dorsiflexion with their most affected side and all controls performed dorsiflexion with their right foot. Second, we examined gait initiation, while subjects were standing 4 meters in front of the LED-arrays. Again, illumination of the first LED-array served as a warning signal, and illumination of the second array as the IS. Participants were instructed to perform rapid gait initiation at the IS, without further instruction about which foot to step with first.

In both tasks, the forewarning periods (1–3,5 seconds) and the inter-trial intervals (6–10 seconds) were variable. All subjects performed 16 dorsiflexion trials and 16 gait initiation trials. In 25% of trials (4 during each task) a SAS was given simultaneously with the IS. The SAS (50 ms white noise, 116 dB sound pressure level) was generated by a custom-made noise generator and delivered through binaural earphones. Prior to each task, subjects were allowed five practice trials.

Data collection

EMG. EMG data were collected from bilateral tibialis anterior (TA) and rectus femoris muscles (RF) and the left sternocleidomastoid (SCM) muscle (ZeroWire by Aurion, Italy). EMG signals were sampled at 2000 Hz, full-wave rectified and low-pass filtered at 30 Hz (zero-lag, second order Butterworth filter).

Motion analysis. Reflective markers were placed using a full-body model.[84] Marker positions were recorded by an 8-camera 3D motion analysis system (Vicon Motion Systems, United Kingdom) at a sample rate of 100 Hz. Furthermore, to determine movement onsets in the ankle dorsiflexion task, we placed a triaxial accelerometer on top of the foot. Accelerometer signals were sampled at 2000 Hz.

Force plates. Ground reaction forces under both feet were recorded by two force plates (60x180 cm each; AMTI Custom 6 axis composite force platform, USA), embedded in the surface. The signals of the force plates were sampled at 2000 Hz and low-pass filtered at 10 Hz (second order Butterworth filter).

Data analysis

Simple reaction time task. Two reaction time parameters were assessed, accelerometer reaction time and EMG-reaction time in the TA. Onset latencies were determined using a semi-automatic computer algorithm that selected the first instant at which the EMG-activity or foot accelerations exceeded a threshold of 2 SD above the mean baseline activity, as calculated over a 500 ms period just prior to the IS.

Gait initiation. The outcomes of the gait initiation task included the onset and amplitude of stepping-leg EMG-activity in the TA and RF. Onset latencies were determined using the aforementioned algorithm. The average EMG response amplitude was calculated over a period of 100 ms following onset latency, after subtraction of average baseline activity.[62,266,293] For each trial, we also determined whether an anticipatory postural adjustment (APA) occurred prior to step onset. A weight shift was considered to be an APA if it met two criteria: first, the difference between the vertical loading underneath the stance and stepping leg had to rise above a threshold of 2 SD above the mean difference, as calculated over a 500 ms period prior to the IS. This moment was defined as the onset of the APA. Second, the increase in force under the stepping leg had to exceed 5% of the total body weight. For each APA, we determined the maximum increase in vertical force under the stepping leg, normalized for body weight. We also determined whether multiple APAs occurred.

Furthermore, we determined step onset and length for each trial separately, using the horizontal displacement of the heel and toe markers.

Startle reflex. For each trial in which a SAS was applied, we determined whether a startle reflex occurred. A startle reflex was defined as a short latency response in the SCM-muscle, starting within 130 ms following the SAS.

Statistical analysis

Differences in the outcomes of the clinical assessment between freezers and non-freezers were tested using unpaired t-tests. Outcome measures of the ankle dorsiflexion and gait initiation task were analyzed using a repeated measures ANOVA, with SAS (*SAS –non-SAS*) as within-subject factor and Group (*freezing–non-freezing–controls*) as between-subjects factor. In case of a significant *SASxGroup* interaction, we used Tukey post-hoc tests to identify differences in SAS-induced effects between the groups. The latter post-hoc test was also performed in the case of significant *Group* interactions.

To identify whether the SAS-effects on muscle onset latencies were independent of bradykinesia, we also conducted these analyses with UPDRS bradykinesia subscores as a covariate. As bradykinesia did not change any of the statistical outcomes, these results are not further reported. The alpha level was set at 0.05.

Results

Clinical assessment

Clinical characteristics of the participants are shown in Table 1. Freezers and non-freezers did not differ with respect to age (t(24)=-0.272, p=0.788), nor did the non-freezers and controls (t(27)=0.103, p=0.919). The total UPDRS-III score, UPDRS-bradykinesia items subscore and FAB-score did not differ significantly between freezers and non-freezers (t(24)=-0.958, p=0.348; t(24)=-0.424; p=0.675 and t(24)=-0.542, p=0.593, respectively). Freezers had a significantly higher score on the N-FOGQ (t(24)=10.846, p<0.001), UPDRS-PIGD-subscore (t(24)=-2.900, p=0.008) and a longer disease duration (t(24)=2.501, p=0.020).

Table 1 Participant characteristics.

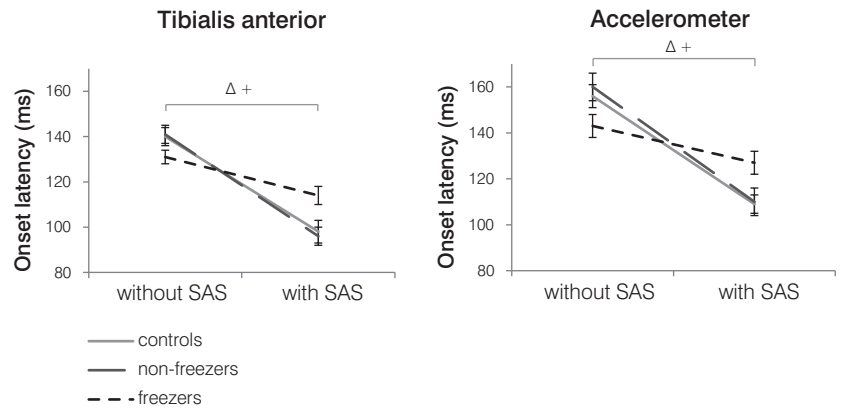
	Freezers	Non-freezers	Controls
Age (years)	68 (60-82)	67 (59-78)	67 (57-77)
Sex	10 M, 2 F	10 M, 4 F	11 M, 4 F
UPDRS-PIGD items	7 (2-13)	4 (0-10)	
UPDRS-bradykinesia items	19 (1-31)	18 (10-28)	
UPDRS-residual items	13 (5-9)	13 (5-24)	
N-FOGQ	17 (9-26)	0.9 (0-9)	
FAB	15 (9-18)	15 (8-18)	
Disease duration (years)	8.9 (2-14)	11.9 (4-23)	

Data are mean (range).
UPDRS = MDS-Unified Parkinson's disease rating scale part III. PIGD-items = postural instability/gait difficulty items (item 9-13; score/20), bradykinesia items (item 4-8 and 14; score/44), residual items (items 1-3 and 15-18; score/68). N-FOGQ = New Freezing of Gait Questionnaire (score/33), FAB = Frontal Assessment Battery (score/18). For both MDS-UPDRS and N-FOGQ, higher scores indicate worse functioning. For FAB, lower scores indicating worse functioning.

Ankle dorsiflexion task

A SAS accelerated the onset of TA-responses (*SAS*; $F_{1,38}$=226.256, p<0.001), but the acceleration differed significantly between the groups (*SASxGroup*; $F_{2,38}$=13.581, p<0.001; Figure 1). The acceleration was attenuated in the freezers (17 ms acceleration to 114±15 ms) compared to the non-freezers (44 ms acceleration to 96±16 ms, p<0.001) and controls (42 ms acceleration to 96±18 ms, p<0.001), whereas non-freezers and controls did not differ from each other (p=0.885). Without a SAS, the onset latencies did not differ between the groups (p>0.175). This pattern was confirmed by the accelerometer data, yielding a significant *SASxgroup* interaction ($F_{2,38}$=11.205, p<0.001; Figure 1), with less acceleration in the freezers.

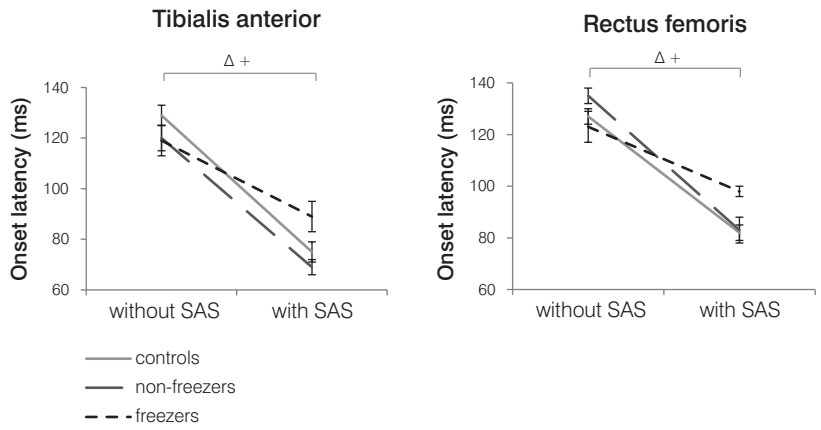
Figure 1 Onset latencies (SE) for the simple reaction time task involving ankle dorsiflexion. + indicates significant SAS interaction, Δ indicates significant SAS x group interaction.



Muscles responses in gait initiation

No FOG-episodes were observed during the gait initiation task. Prior to step onset, we observed consistent activation of TA in the stepping leg to initiate the APA, as well as activation of RF in the vast majority of the participants (37/41). A SAS accelerated the onset of TA-response (*SAS*; $F_{1,38}$=284.554, p<0.001; Figure 2), but this effect differed significantly between groups (*SAS x Group*; $F_{2,38}$=7.030, p=0.003). The acceleration was less pronounced in the freezers (31 ms acceleration to 88±119 ms) compared to the non-freezers (51 ms acceleration to 69±13 ms, p=0.012) and controls (54 ms acceleration to 75±15 ms, p=0.003), whereas non-freezers and controls did not differ from each other (p=0.894). Without a SAS, the onset latencies of TA-responses did not differ between the groups (p>0.332).

Figure 2 Onset latencies of muscle responses involved in the anticipatory postural adjustments (APAs) prior to gait initiation. Mean latencies (SE) are shown for tibialis anterior(left panel) and rectus femoris (right panel) of the stepping leg. + indicates significant SAS interaction, Δ indicates significant SAS x group interaction.



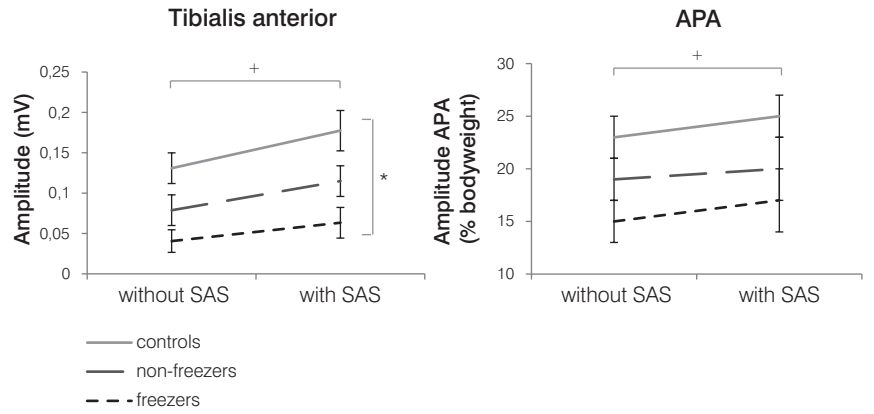
The same pattern of results was found for RF onset latencies (*SASxgroup*; $F_{2,34}$=4.771, p=0.015; Figure 2). A smaller SAS-induced acceleration was seen in the freezers (25 ms acceleration to 98±33 ms) compared to the non-freezers (52 ms acceleration to 83±19 ms, p=0.012) and controls (45 ms acceleration to 82±12 ms, p=0.068). Without a SAS, there were no between-group differences in RF onset latencies (p>0.136).
The SAS increased the amplitude of TA-responses by 41% (*SAS;* $F_{1,38}$=18.503, p<0.001). This effect did not differ between the groups (*SASxGroup*; $F_{2,38}$=0.689, p=0.508; Figure 3). There was, however, a significant group effect (G*roup*; $F_{2,38}$=7.168, p=0.002), with smaller overall TA-responses in freezers compared to controls (p=0.004).
The SAS increased the amplitude of RF-responses by 40% (*SAS;* $F_{1,34}$=9.184, p=0.005), without differential group effects (*SASxGroup*; $F_{2,34}$=0.274, p=0.762; G*roup*; $F_{2,34}$=0.464, p=0.632).

Anticipatory adjustments in gait initiation

APAs were detected in more than more than 90% of trials, irrespective of group or SAS. We did not record any multiple APAs, which is in line with the absence of FOG-episodes during the experiment.
The SAS significantly accelerated APA-onsets (*SAS;* $F_{1,38}$=167.692, p<0.001), but this effect differed between groups (*SASxGroup*; $F_{2,38}$=7.245, p=0.002). The acceleration was less pronounced in freezers (34 ms acceleration to 160±60 ms) compared to non-freezers (73 ms acceleration to 119±24 ms, p=0.003) and controls (68 ms

acceleration to 125±26 ms, p=0.010), whereas non-freezers and controls did not differ from each other (p=0.885). In trials without a SAS, APA-onset did not differ between the groups (p>0.997).
The SAS increased APA-amplitude by on average 10% (*SAS*; $F_{1,38}$=4.722, p=0.036; Figure 3), and this effect did not differ between the groups (*SASxGroup*; $F_{2,38}$=0.061, p=0.941). Although the APA-amplitude tended be smaller in freezers compared to non-freezers and controls, and smaller in non-freezers compared to controls, the group effect did not reach significance (*Group*; $F_{2,38}$=3.012, p=0.061).

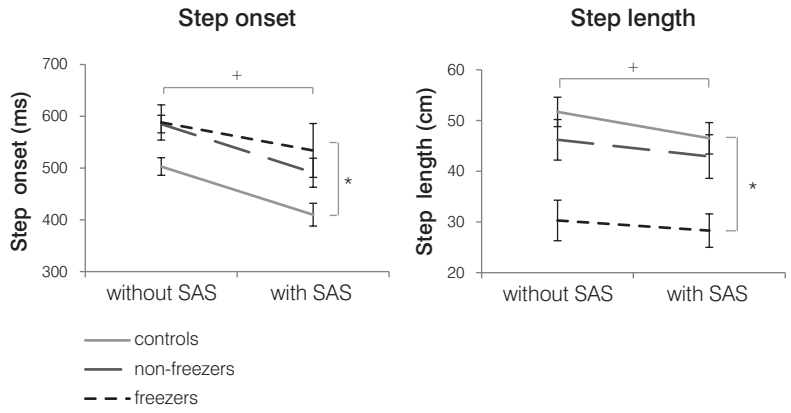
Figure 3 Mean amplitudes (SE) of stepping-leg tibialis anterior responses (left panel) and anticipatory postural adjustments (APAs; right panel) prior to gait initiation. + indicates significant SAS interaction, * indicates significant group interaction.



Step onset and length in gait initiation

The SAS accelerated step onset (*SAS*; $F_{1,38}$=64.430, p<0.001; Figure 4). The effect of the SAS did not differ between the groups (*SASxGroup*; $F_{2,38}$=1.697, p=0.197), although the acceleration tended to be smaller in freezers (54 ms acceleration) compared to non-freezers (94 ms) and controls (93 ms). There was a significant group effect (*Group*; $F_{2,38}$=4.012, p=0.026). Without a SAS, step initiation was delayed in freezers (588±119 ms) and non-freezers (585±64 ms) compared to controls (503±65 ms; p=0.032 and p=0.034, respectively), whereas step onset did not differ between freezers and non-freezers (p=0.997).
The SAS shortened the length of the first step by on average 4 cm (*SAS*; $F_{1,38}$=11.747, p=0.001; Figure 4), which effect did not differ between the groups (*SASxGroup*; $F_{2,38}$=0.797, p=0.458). Step lengths differed between groups (*Group*; $F_{2,38}$=8.089,

Figure 4 Mean (SE) step onset and step length of the first step during gait initiation. + indicates significant SAS interaction, * indicates significant group interaction.



p=0.001) with shorter steps in freezers (30±14 cm) compared to non-freezers (46±15 cm; p=0.013) and controls (52±11 cm; p=0.001). Step lengths did not differ between non-freezers and controls (p=0.531).

Startle reflexes

In the gait initiation task, we found no differences in startle reflex occurrence between freezers (31% of trials with SAS), non-freezers (25% of trials with SAS) and controls (33% of trials with SAS, $F_{2,40}$=0.178, p=0.838). This pattern was confirmed by the ankle dorsiflexion task, where no difference in startle reflex occurrence was seen between freezers (25% of trials with SAS), non-freezers (27% of trials with SAS) and controls (38% of trials with SAS, $F_{2,40}$=0.464, p=0.632). Furthermore, a higher occurrence of startle reflexes was not associated with a larger StartReact effect, neither in the gait initiation task (r_p=0.146, p=0.362), nor in the ankle dorsiflexion task (r_p=0.167, p=0.297).

Discussion

We found that the accelerating effect of a startling auditory stimulus (SAS) was attenuated in PD-patients with FOG, and this was seen for both gait initiation and for a simple reactive ankle dorsiflexion movement. The SAS-induced accelerations were independent of the occurrence of startle reflexes in the sternocleidomastoid muscle. The reduced StartReact effect differentiated freezers from non-freezers with similar

disease severity, whereas non-freezers did not differ from control subjects with regard to the effects of the SAS; this result was independent of severity of bradykinesia. Furthermore, freezers had reduced step lengths of their first step to initiate gait.

Deficient StartReact effect in freezers

The present study is the first to apply the StartReact paradigm to gait initiation in PD-patients with FOG, providing strong evidence for the coexistence of freezing and reduced StartReact effects. We were able to confirm the disturbed StartReact effect in freezers during simple reactive movements, shown previously for an upper limb task,[349] now replicated for a simple ankle dorsiflexion movement. Importantly, the present results extend these previous findings in three ways. First, we show that attenuation of the StartReact effect is not restricted to simple movements, but also occurs in gait initiation, a complex whole-body movement that can provoke freezing episodes. Second, the present results were obtained in a less severely affected group of patients with predominantly OFF-period FOG, who are more representative of 'typical' PD-patients compared to the group with severe ON-period freezing that was included by Thevathasan et al.[349] Third, we included patients without prior PPN surgery, which allowed us to study the presumed StartReact effects without the possible influence of surgical microlesions or chronic after-effects of DBS.

We observed that in non-freezers, the SAS accelerated the EMG and movement onsets to the same extent as in controls. This confirms previous observations on simple reactive movements as well as gait initiation in PD.[61,119,307] Apparently, the pre-programming of motor responses and their reflexive release by the SAS is still intact in these patients. In contrast, PD patients with FOG showed a consistently attenuated StartReact effect. The pmRF presumably plays a pivotal role in the StartReact effect.[271,359] Hence, we suggest that in freezers, motor responses (including the APA to initiate a step) may be poorly represented in this brainstem reticular structure, or that the reflexive release of these motor responses may be deficient due to pmRF networks that encode the motor response being less responsive to excitatory stimuli. The latter could be the result of enhanced inhibitory drive from other structures, likely involving the PPN, as it has strong inhibitory projections on the pmRF.[174,193,298] This notion is coherent with the reported effects of PPN-stimulation on StartReact effects.[349]

Underscaling of gait parameters

In the current study, we confirmed the underscaling of step length in freezers that was observed previously.[68,256] The underscaling of step length was independent of the presence of a SAS. Interestingly, a SAS did result in a small but significant reduction of step length, both in PD patients and in controls. The mechanisms underlying the reduction of step length by a SAS are not clear, and should be explored by future

studies. In addition to the underscaling of step length, both freezers and non-freezers had a tendency for smaller amplitudes of anticipatory postural adjustments (APAs) compared to controls. This tendency is in line with the previously reported underscaling of APAs in PD.[46,77,131,232,307,362] Both the reduced step length and the smaller APAs have been attributed to reduced brain activity in the supplementary motor area (SMA)[148,329] and are thought to contribute to FOG.[68] It is conceivable that the mechanisms underlying the underscaling of movements are different from those underlying the deficient StartReact effect, as non-freezers showed underscaling APAs as well, but still exhibited an intact StartReact effect. Furthermore, freezers demonstrated intact augmentation of EMG-response amplitudes due to the SAS, but at the same time exhibited consistent delays in the onset of these responses.

Relation between disturbed StartReact and freezing of gait

The finding of attenuated SAS-induced accelerations of motor responses in freezers raises the question whether it may be relevant to the causation of FOG. The neural structures most likely involved in the StartReact phenomenon (pmRF and PPN) are also thought to be involved in the integration of APAs with subsequent stepping movements.[199,246,275,317] The results of our gait initiation task point at deficiencies in APA-representation or release at brainstem level, which may compromise the integration with subsequent steps. This possibly leads to further underscaling and increased variability in step lengths, as previously reported in freezers.[68,152,256] With time-varying demands such as turning, or when exaggerating the underscaling of gait characteristics (e.g. when making small steps), these spatiotemporal gait abnormalities increase the computational load on the PPN and the pmRF. At such instances, these structures may no longer be able to coordinate the integration of APAs with steps, leading to FOG-episodes. As this hypothesis remains speculative and largely based on indirect evidence, further studies are needed to corroborate whether brainstem structures are indeed unable to integrate the different motor programs during a FOG-episode, for example by directly measuring the oscillatory activity of the PPN during a FOG-episode. A first report has already associated attenuation of PPN alpha activity with FOG,[351] but this promising finding warrants further investigation.

9

StartReact effects support different pathophysiological mechanisms underlying freezing of gait and postural instability in Parkinson's disease

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Abstract

The pathophysiology underlying postural instability in Parkinson's disease is poorly understood. The frequent co-existence with freezing of gait raises the possibility of shared pathophysiology. There is evidence that dysfunction of brainstem structures contribute to freezing of gait. Here, we evaluated whether dysfunction of these structures contributes to postural instability as well. Brainstem function was assessed by studying the StartReact effect (acceleration of latencies by a startling acoustic stimulus (SAS)). We included 25 patients, divided in two different ways: 1) those with postural instability (HY=3, n=11) versus those without (HY<3, n=14); and 2) those with freezing (n=11) versus those without freezing (n=14). We also tested 15 matched healthy controls. We tested postural responses by translating a balance platform in the forward direction, resulting in backward balance perturbations. In 25% of trials, the start of the balance perturbation was accompanied by a SAS.

The amplitude of automatic postural responses and length of the first balance correcting step were smaller in patients with postural instability compared to patients without postural instability, but did not differ between freezers and non-freezers. In contrast, the StartReact effect was intact in patients with postural instability but was attenuated in freezers.

We suggest that the mechanisms underlying freezing of gait and postural instability in Parkinson's disease are at least partly different. Underscaling of automatic postural responses and balance-correcting steps both contribute to postural instability. The attenuated StartReact effect was seen only in freezers and likely reflects inadequate representation of motor programs at upper brainstem level.

Introduction

Postural instability is a disabling feature of Parkinson's disease (PD), in which the underlying pathophysiology is still poorly understood. The frequent co-existence with freezing of gait (FOG) raises the possibility of a shared pathophysiology.[132,179] There is emerging evidence that dysfunction of upper brainstem structures, in particular the pedunculopontine nucleus (PPN) and pontomedullary reticular formation (pmRF), could play a role in causing FOG.[271,275,349] As automatic postural responses likely arise from the pmRF,[337] dysfunction of upper brainstem structures may also underlie postural instability.

Evidence for a pivotal role of dysfunctional upper brainstem circuits in patients with FOG has been provided by studies evaluating the StartReact effect. StartReact refers to the acceleration of movement onset latencies when a startling auditory stimulus (SAS) is given at the same time as the imperative 'go' signal in a reaction time task. Although the exact mechanism underlying StartReact and the neural structures involved are a matter of ongoing debate,[3,271] several recent studies have provided accumulating evidence for the SAS directly releasing a subcortically stored motor program, presumably from upper brainstem structures.[55,125,162,165,271,359] The StartReact effect was absent in patients with severe FOG and postural instability when performing an elbow flexion movement, but was restored after PPN stimulation.[349] In a recent study we further confirmed that the StartReact effect is attenuated in freezers, but -more importantly- in a task that is known to provoke FOG (i.e. gait initiation).[269] Postural responses to backward balance perturbations can be modified by a StartReact paradigm,[266] suggesting that they are preprogrammed and potentially subject to the same triggered release which has been shown to be deficient in freezers.[349] However, SAS-induced acceleration of postural responses has not been evaluated in PD patients. Moreover, neither Thevathasan *et al.* nor Nonnekes *et al.* evaluated whether defective StartReact effects are also related to postural instability. Therefore, it remains unknown whether dysfunction of the same brainstem reticular structures may underlie both FOG and postural instability.

In the present study, we aimed to address this question by evaluating the effect of a SAS on the onset and scaling of postural responses to backward balance perturbations. As a control, we also evaluated SAS-induced movement accelerations in a simple reaction time task involving ankle dorsiflexion. We carefully selected and balanced a group of PD patients, and specifically contrasted the results between patients with evident postural instability (as identified by a positive pull test) versus those without, and between those with FOG versus those without. If dysfunction of the same brainstem reticular structures contributes to both FOG and postural instability, we should expect to see a disturbed StartReact effect in freezers as well as in patients with postural instability.

Materials and methods

Participants

The present paper concerns the same participants as in our previous paper,[269] except for one PD patient for whom the examination of balance responses was too burdensome. The PD group consisted of twenty-five patients, 11 with evident postural instability (Hoehn and Yahr stage 3) and 14 without evident postural instability (Hoehn and Yahr stage 2 or 2.5). Six of the patients with postural instability had freezing of gait, and this was also true for 5 of the patients without postural instability (see below for definitions). Patients were diagnosed according to the UK Brain Bank criteria.[172] Exclusion criteria were any other neurological or orthopedic disorder affecting balance, severe cognitive impairments or use of medication negatively affecting balance. Patients were measured in an OFF state, when they experienced an end-of-dose effect prior to intake of their next medication. Clinical assessment also took place in the OFF state. In addition, 15 healthy controls of similar age were included. The study was approved by the local medical ethics committee (CMO regio Arnhem/Nijmegen) and was conducted in accordance with the Declaration of Helsinki. All subjects gave their written informed consent prior to the experiment.

Clinical assessment

PD patients were clinically assessed with the motor subsection (Part III) of the MDS-Unified Parkinson's Disease Rating Scale (UPDRS, score/132).[137] Postural instability was determined by the pull-test performed by JN. The pull-test was performed as described in the MDS-UPDRS,[137] which is regarded as the gold standard to evaluate postural instability in PD.[151,272] A mild pull was applied first, which was not rated and served as a demonstration. Thereafter, a quick and forceful pull was applied, which was rated. Patients with Hoehn and Yahr stage 3 were unable to recover independently, and would have fallen if not caught by the examiner. Patients with Hoehn and Yahr stage<3 were able to recover unaided. Patients also completed the New Freezing of Gait Questionnaire (N-FOGQ, score/33).[258] Additionally, they performed a series of walking tests to objectively verify subjects as freezers or non-freezers.[331,332] These tests included eight rapid axial 360° turns in both directions and walking with 25% of the preferred step length (at a normal pace, and as rapidly as possible). Based on the detailed physical examination, 11 persons were classified as 'freezers', and the 14 other patients were classified as 'non-freezers' as they did not show FOG-episodes during examination, and never experienced subjective gluing in daily life (except for one patient with sporadic gluing in daily life, but who never manifested FOG during repeated and detailed neurological examinations). The N-FOGQ revealed that all freezers had more frequent and more severe FOG during the OFF-medication state. Lastly, global executive function was assessed with the Frontal Assessment Battery (FAB, score/18).

Experimental setup and protocol

Postural responses Participants stood on a moveable platform that could suddenly translate in the forward direction, resulting in a backward balance perturbation; we will refer to the direction of the balance perturbation.[264] Participants stood with their arms alongside the trunk. Platform movements comprised an acceleration phase (300 ms), a constant velocity phase (500 ms), and a deceleration phase (300 ms). We used perturbations with an acceleration of 1.5 m/s^2. This intensity required all participants taking one or more steps to prevent falling. Participants were instructed to respond to the balance perturbations as they would do in daily life. Participants underwent 16 backward balance perturbations and consecutive trials were at least 20 seconds apart. In 25% of the balance perturbations a startling auditory stimulus (SAS) was given through binaural earphones at the start of the translation of the platform. The SAS (50 ms white noise, 116 dB sound pressure level linear fast (measured with Investigator 2260 and Artificial Ear B&K 6cc type 4152, Bruel and Kjaer, Nærum, Denmark)) was generated by a custom-made noise generator and was randomized over the trials. Subjects wore a safety harness that was attached to the ceiling and prevented them from falling.

Simple reaction time task This task served to verify whether the pattern of results in the postural perturbations would also apply to a different type of movement. The results of the freezers versus non-freezer comparison have been reported in our previous paper,[269] but here we will also compare patients with and without postural instability. The task involved subjects performing a simple reactive ankle dorsiflexion movement. Participants sat in a chair that was positioned 2.5 meters in front of two arrays of light-emitting diodes (LEDs; 11x8 cm, 3 cm apart). Illumination of the first LED array served as a warning signal and participants were instructed to initiate ankle dorsiflexion as soon as the second LED array was lit ('go' signal). Patients performed the task with their most affected side and healthy controls with their right foot. Warning periods (1 – 3.5 seconds) and inter-trial periods (6 – 10 seconds) varied. All participants performed 16 trials. In 25% of trials a SAS was given at the instant of the 'go' signal.

Data collection

EMG. EMG data were collected from the tibialis anterior and rectus femoris muscles on both sides of the body, and the left sternocleidomastoid (SCM) muscle. Self-adhesive Ag-AgCl electrodes (Tyco Arbo ECG) were placed approximately 2 cm apart and longitudinally on the belly of each muscle, according to Seniam guidelines[156] EMG signals were sampled at 2000 Hz, and full-wave rectified and low-pass filtered at 30 Hz (zero-lag, second order Butterworth filter).

Motion analysis. To evaluate the postural responses, reflective markers were placed using a full-body model.[84] Marker positions were recorded by an 8-camera 3D motion

analysis system (Vicon Motion Systems, United Kingdom) at a sample rate of 100 Hz and low-pass filtered at 10 Hz (zero-lag, second order Butterworth filter). During the simple reaction time task, a triaxial accelerometer was placed on the foot that performed the ankle dorsiflexion movement. Accelerometer signals were sampled at 2000 Hz.

Data analysis

Postural responses. For each participant, the ensemble average EMG activity during trials was calculated for each muscle, separately for trials with and without a SAS. Onset latencies of tibialis anterior and rectus femoris activity (the prime movers for the evoked postural response) were determined using the semi-automatic computer algorithm that selected the first instant at which the EMG activity exceeded a threshold of 2 standard deviations above the mean background activity, as calculated over a 500 ms period just prior to platform movement. Latencies were first selected by the computer algorithm, then visually approved or (when necessary) corrected.[266] Mean response amplitude of the ensemble average was calculated over 100 ms following the onset of muscle activity and corrected for background EMG activity. The mean onset and amplitude of tibialis anterior and rectus femoris activity in the left and right leg was taken, as there was no systematic difference between the legs; either when comparing the left and right leg, or when comparing the most and least affected leg. Step onset and step length were determined using the position data of the heel and toe markers. Step onset was defined as the time between the start of the platform displacement and the time at which the heel and toe markers moved backwards with respect to the platform (velocity > 0,1 m/s). Step length was defined as the backward displacement of the toe markers during the step. We determined the number of balance correcting steps by visual inspection of video data.

To determine the 'quality' of the first balance correcting step, we calculated the angle of the stepping leg at the end of the first step (i.e. foot contact of the stepping leg).[373] The leg angle is the angle of the line connecting the toe marker and the midpoint of the pelvis markers with respect to the vertical. A negative leg angle during backward stepping represents a situation in which the pelvis is located posterior to the stepping foot. Thus, following backward perturbations a more negative leg angle represents a more inefficient first step.

Startle reflex. For each trial in which a SAS was applied, we determined whether a startle reflex occurred. A startle reflex was defined as a short latency response in the SCM-muscle, starting within 130 ms following the SAS. The response had to exceed, for at least 20 ms, a threshold of 2 SD above mean background activity, as calculated over a 500 ms period just prior to the SAS.*Simple reaction time task.* Two reaction time parameters were assessed, accelerometer reaction time and EMG reaction time

in tibialis anterior muscle. Onset latencies of EMG activity and foot accelerations were determined using a semi-automatic computer algorithm described above.

Statistical analysis

Data from PD patients were analyzed using a repeated measures ANOVA, with SAS (*SAS –no SAS*) as within subjects factor and HY-stage (*HY<3 – HY3*) and freezing (*freezing – non-freezing*) as between- subjects factors. In case of a significant *SASxHY-stage* or *SASxfreezing* interaction, post-hoc Student's t-tests were performed to identify differences between subgroups.

To determine whether outcomes differed between patients and control subjects, independent of clinically-identified postural instability or freezing of gait, we compared the controls with the least affected patients (either HY<3 or non-freezers). To this aim, we performed a repeated measures ANOVA, with SAS as within subjects factor and group (*controls – least affected patients*) as between subjects factor.

Finally, in PD patients, we determined Pearson's correlation coefficients for non-startle trials between the amplitude of tibialis anterior activity during the postural responses and (i) the step length and (ii) the leg angle. The alpha level was set at 0.05.

Results

Clinical assessment

Clinical characteristics of the study participants are shown in Table 1. Patients with postural instability were on average six years older than those without postural instability ($t(23)=-2.186$, $p=0.039$); age did not differ between freezers and non-freezers ($t(23)=0.173$, $p=0.864$). The MDS-UPDRS-III score did not differ significantly between patients with and without postural instability ($t(23)=-1.003$, $p=0.326$), nor between freezers and non-freezers ($t(23)=-0.615$, $p=0.544$). In addition, the FAB-score did not differ between the subgroups ($t(23)<0.768$, $p>0.450$). Freezers had higher scores on the N-FOGQ compared to non-freezers ($t(23)=-11.296$, $p<0.001$); the N-FOGQ score did not differ between patients with and without postural instability ($t(23)=-0.635$, $p=0.532$).

Automatic postural response

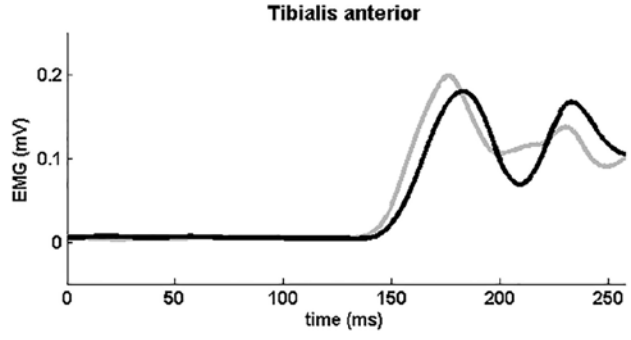
A backward perturbation always resulted in a bilateral response in the tibialis anterior and rectus femoris muscles. The SAS accelerated the onset of the tibialis anterior responses in PD patients by on average 14 ms (*SAS*; $F_{1,21}=13.633$, $p=0.001$; Figure 1 and 2). Latencies and their acceleration by the SAS did not differ between patients with and without postural instability (*SASxHY-stage*; $F_{1,21}=0.173$; $p=0.681$). However, the acceleration of tibialis anterior responses was significantly attenuated in the

Table 1 Participant characteristics.

	Age (years)	Sex	UPDRS-III	No. of freezers	N-FOGQ	FAB	Disease duration (years)
HY<3	64 (55-76)	12 M, 2 F	35 (14-50)	5	7 (0-22)	15 (9-18)	10 (4-23)
HY3	70 (59-81)	8 M, 3 F	38 (23-50)	6	8 (0-22)	14 (8-18)	10 (2-16)
controls	67 (57-77)	11 M, 4 F					

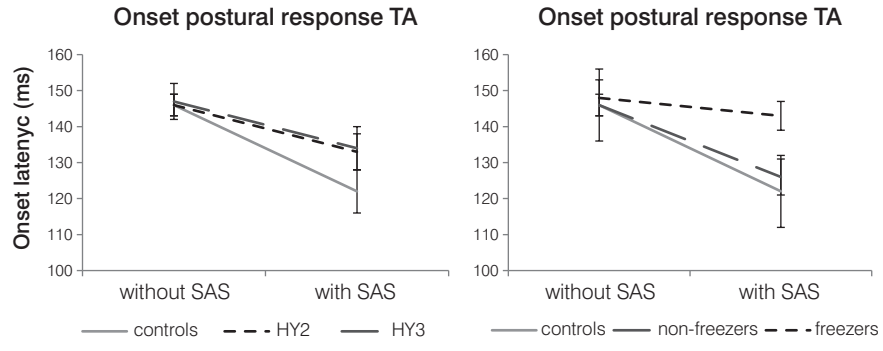
Data are mean (range).
UPDRS =MDS-Unified Parkinson's disease rating scale part III (score/132), N-FOGQ = New Freezing of Gait Questionnaire(score/33), FAB = Frontal Assessment Battery (score/18)

Figure 1 Average EMG-activity recorded in the tibialis anterior muscle of a single PD-patient (with freezing of gait and postural instability) during backward balance perturbations. Grey line represents perturbations with SAS (determined onset latency = 140 ms). Black line represents perturbations without SAS (determined onset latency = 145 ms).



freezers (5 ms acceleration) compared to the non-freezers (20 ms acceleration; *SASxfreezing*; $F_{1,21}$=5.150, p=0.034; Figure 2). Post-hoc analysis revealed that latencies during trials without a SAS did not differ between freezers and non-freezers (t(23)=-0.391, p=0.699), whereas with a SAS, they were significantly delayed in the freezers compared to non-freezers (t(23)=-2.447, p=0.022). Non-freezers did not differ from controls (*Group*; $F_{1,27}$=0.107; p=0.746; *SASxGroup*; $F_{1,27}$=0.210, p=0.651). The same pattern was seen for the rectus femoris muscle. The SAS accelerated the onset of the postural responses in PD patients by on average 10 ms (*SAS*; $F_{1,21}$=10.743, p=0.004). The acceleration did not differ significantly between patients with and without postural instability (*SASxHY-stage*; $F_{1,21}$=1.247; p=0.277), but was significantly

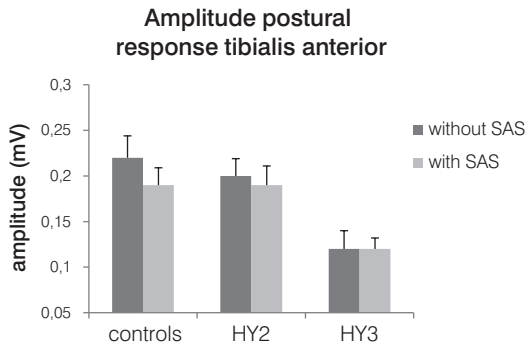
Figure 2 Mean onset latencies (SE) of the automatic postural response in tibialis anterior (TA). HY = Hoehn and Yahr stage. A SAS significantly accelerated automatic postural responses. Latencies and their acceleration by the SAS did not differ between patients with and without postural instability. The SAS-induced acceleration of tibialis anterior responses was significantly attenuated in the freezers compared to the non-freezers. Non-freezers did not differ from controls.



reduced in the freezers (2 ms acceleration) compared to the non-freezers (14 ms acceleration; *SASxFreezing*; $F_{1,21}$=6.473, p=0.019). Post-hoc analysis revealed that latencies during trials without a SAS did not differ between freezers and non-freezers (t(23)=-1.439, p=0.164), whereas they were significantly delayed in the freezers compared to non-freezers following SAS presentation (t(23)=-2.416, p=0.043). Non-freezers did not differ from controls (158±13, 18 ms acceleration, *Group*; $F_{1,27}$=0.013; p=0.909; *SASxGroup*; $F_{1,27}$=0.544, p=0.467).

The SAS had no effect on the amplitudes of tibialis anterior or rectus femoris activity (*SAS*; $F_{1,21}$=1.105, p=0.305; *SAS*; $F_{1,21}$=2.122, p=0.160, respectively). Tibialis anterior amplitudes were on average 40% smaller in patients with postural instability compared to patients without postural instability (*HY-stage*; $F_{1,21}$=7.308, p=0.013; Figure 3), whereas they did not significantly differ between freezers and non-freezers (*Freezing;* $F_{1,21}$=2.963, p=0.100). Rectus femoris amplitudes were on average 21% smaller in patients with postural instability compared to patients without postural instability, but this difference did not reach significance due to large within- and between-subjects variability (*HY-stage*; $F_{1,21}$=0.588, p=0.452). Rectus femoris amplitudes did not differ between freezers and non-freezers either (*Freezing*; $F_{1,21}$=0.159, p=0.694). In addition, amplitudes of tibialis anterior and rectus femoris responses did not differ between patients without postural instability and controls (*Group*; $F_{1,27}$=0.122; p=0.729; *Group*; $F_{1,27}$=1.634; p=0.212, respectively).

Figure 3 Mean amplitudes (SE) of the automatic postural response in tibialis anterior (TA). HY = Hoehn and Yahr stage. Tibialis anterior amplitudes were significantly smaller in patients with postural instability compared to patients without postural instability, whereas they did not significantly differ between freezers and non-freezers. Amplitudes of tibialis anterior did not differ between patients without postural instability and controls.



Balance correcting step

Step onset did not differ between patients with and without postural instability (*HY-stage*; $F_{1,21}$=0.001, p=0.971; Table 2), nor between freezers and non-freezers (*Freezing*; $F_{1,21}$=0.079, p=0.782). The SAS had no general effect on the step onset (*SAS*; $F_{1,21}$=0.988, p=0.332). In the freezers, however, we observed later step onsets in trials with a SAS, whereas non-freezers demonstrated an earlier step onset, yielding a significant *SASxFreezing* interaction ($F_{1,21}$=6.614, p=0.018). Step onset did not differ between non-freezers and controls (*Group;* $F_{1,27}$=0.007, p=0.936).

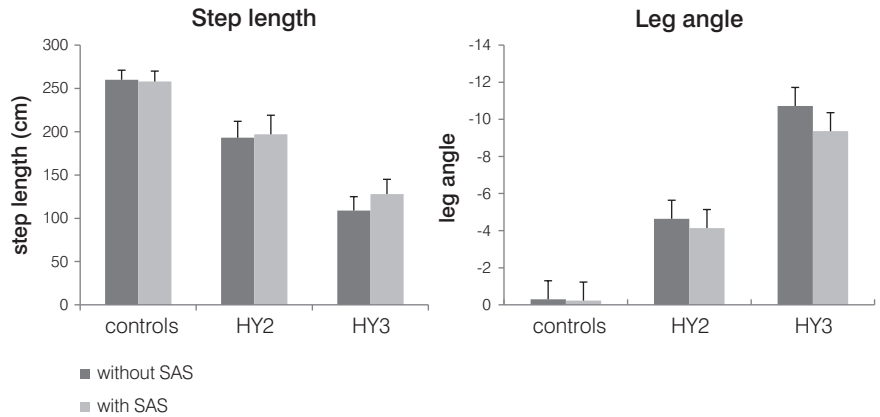
Table 2 Step onset and number of balance correcting steps.

	Step onset (ms)		Number of steps	
	No SAS	SAS	No SAS	SAS
Controls	349±51	337±45	1.3±0.4	1.3±0.4
HY<3	347±53	340±57	1.6±0.6	1.8±0.7
HY3	351±45	345±58	2.5±0.8	2.3±0.6
freezers	337±57	363±51	2.4±0.8	2.3±0.7
non-freezers	346±58	337±46	1.7±0.7	1.8±0.6

Values are mean (SD).

Patients with postural instability had smaller step lengths (12±5 cm) than patients without postural instability (20±7 cm; *HY-stage*; $F_{1,21}$=6.815, p=0.016; Figure 4), but step length did not differ between freezers and non-freezers (*Freezing*; $F_{1,21}$=2.810, p=0.109; Table 2). A SAS did not influence step length (*SAS*; $F_{1,21}$=2.537, p=0.126). Step lengths were shorter in patients without postural instability compared to controls (26±4 cm; *Group;* $F_{1,27}$=8.261; p=0.008; Figure 4).

Figure 4 Mean step lengths and leg angles (SE) during backward perturbations. Patients with postural instability had significantly smaller step lengths than patients without postural instability, but step length did not differ between freezers and non-freezers. Step lengths were significantly shorter in patients without postural instability compared to controls. Leg angles were significantly smaller in patients with postural instability compared to patients without postural instability. Leg angles did not differ between freezers and non-freezers. Patients without postural instability had more negative leg angles compared to controls.



The quality of the balance correcting step was lower in patients with postural instability compared to patients without postural instability as evidenced by more negative leg angles (-10.7±5.0^0 vs -4.6±3.9^0; *HY-stage*; $F_{1,21}$=7.060; p=0.015; Figure 4). Leg angles did not differ between freezers and non-freezers (*Freezing*; $F_{1,21}$=1.602, p=0.219). The SAS improved the leg angle in PD patients by on average 0.9^0 (*SAS*; $F_{1,21}$=10.121, p=0.004; Figure 4) with no differences between patients with and without postural instability (*SASxHY-stage*; $F_{1,21}$=1.757, p=0.199) or between patients with and without freezing of gait (*SASxFreezing*; $F_{1,21}$=0.102, p=0.753). Patients without postural instability had more negative leg angles compared to controls (*Group*; $F_{1,27}$=11.884, p=0.002; Figure 4).

Patients with postural instability needed more steps to recover from the balance perturbations than patients without postural instability (*HY-stage*; $F_{1,21}$=4.765,

p=0.041; see Table 2). The average number of balance correcting steps tended to be higher in freezers compared non-freezers, but differences were not significant (*Freezing*; $F_{1,21}$=3.920, p=0.061). The SAS did not influence the number of steps (*SAS*; $F_{1,21}$=0.830, p=0.373). Patients without postural instability made more steps compared to control subjects (*Group*; $F_{1,27}$=4.343, p=0.047).
In PD patients, the leg angle correlated strongly with step length (r_p=0.887; p<0.001) and moderately with response amplitudes in tibialis anterior (r_p=0.444; p=0.026). Correlations between step length and tibialis anterior amplitudes bordered significance (r_p=0.377; p=0.063).

Correlation between StartReact effects and underscaling

In PD patients, SAS-induced acceleration of postural responses in the tibialis anterior muscle did not correlate with the amplitude of tibialis anterior activity (r_p=0.026; p=0.902), nor with step length (r_p=-0.078; p=0.711) or leg angle (r_p=-0.052; p<0.806).

Association between startle reflexes and StartReact

Following balance perturbations with a SAS, we found no difference in startle reflex occurrence between freezers (23% of trials with SAS), non-freezers (38%), and controls (23%; $F_{2,39}$=0.504, p=0.608). Furthermore, more frequent occurrence of startle reflexes was not associated with a larger StartReact effect in individual participants, neither in tibialis anterior (r_p=0.194, p=0.230) nor in rectus femoris (r_p=0.045, p=0.784). To further investigate the relation between the presence of SCM-reflexes and onset latencies in the TA-muscles during SAS-trials, we determined the onset of TA-responses for each SAS-trial separately. We conducted an ANOVA to compare SAS-induced accelerations in TA onsets between trials with and without SCM reflex. We included g*roup (freezers – non-freezers – controls*) as a between-subjects factor. As participants could either have SCM+ trials only, SCM- trials only or a combination of both, we also included the presence of *SCM reflex (yes/no)* as a between-subjects factor. This analysis demonstrated that overall, accelerations in TA onset latencies did not differ between trials with and without SCM activation (13±4 ms vs. 16±4 ms; *SCM reflex,* $F_{1,49}$=0.321, p=0.573); g*roup x SCM reflex*, $F_{2,49}$=0.280, p=0.757; see Figure 5). Post-hoc LSD tests confirmed the reduced SAS-induced acceleration in the freezers compared to the non-freezers (p=0.043), as well as the absence of differences between non-freezers and controls (p=0.794).

Simple reaction time task

In the ankle dorsiflexion task, the StartReact effect was significantly attenuated in the freezers compared to the non-freezers, which was reflected both in the latencies of tibialis anterior activity (20 ms acceleration for freezers, 45 ms for non-freezers; *SASxFreezing*; $F_{1,21}$=25.651, p<0.001, see Table 3) and in the accelerometer onset

Figure 5 Mean acceleration (SE) of onset latencies of automatic postural responses in tibialis anterior (TA) during SAS-trials with and without a startle reflex in the sternocleidomastoid (SCM) muscle. The number of participants who showed trials with and without SCM reflexes is indicated on top of each bar. In all groups, acceleration of responses did not differ significantly between SAS-trials with and without a startle reflex in the SCM-muscle.

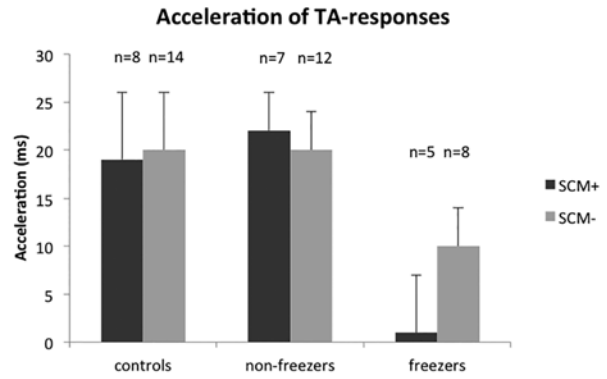


Table 3 Ankle dorsiflexion.

	Onset TA (ms)		Onset accelerometer (ms)	
	No SAS	SAS	No SAS	SAS
Control subjects	140±16	98±18	156±22	109±16
HY<3	135±15	98±18	151±24	112±25
HY3	138±13	108±12	155±20	123±11
freezers	131±11	111±12	144±17	126±15
non-freezers	141±14	96±16	160±24	110±22

Values are mean (SD). HY = Hoehn and Yahr stage.

(18 ms acceleration for freezers, 50 ms for non-freezers; *SASxFreezing*; $F_{1,21}$=13.413, p<0.001). There were no differences in acceleration between patients with and without postural instability, neither in the EMG responses (*SASxHY-stage*; $F_{1,21}$=0.133, p=0.719) nor in the accelerometer onset (*SASxHY-stage*; $F_{1,21}$<0.001, p=0.999).

Discussion

We examined postural responses with and without a startling acoustic stimulus (SAS) in a carefully selected group of PD patients, and specifically contrasted the results between patients with pronounced postural instability versus those without, and between those with FOG versus those without, while statistically controlling for the potential confounding effects of the other factor. Using this method, we were able to delineate characteristics specific to postural instability, and factors specific to freezing of gait. The results of the present study reveal a distinct dissociation between postural instability and FOG. We found reduced amplitudes of automatic postural responses following a backward perturbation, as well as reduced length and quality of the first balance correcting step, in patients with postural instability compared to patients without postural instability. These parameters did not differ between freezers and non-freezers. In contrast, the accelerating effect of a SAS on both postural responses and simple ankle dorsiflexion movements was not different between patients with and without postural instability. Instead, this effect was selectively attenuated in the freezers, whereas it was completely intact in non-freezers. The dissociation between postural instability and FOG was also evident from the lack of associations between StartReact effects and underscaling of balance correcting responses.

Different mechanisms underlie freezing and postural instability

The frequent co-existence of freezing of gait and postural instability has raised the possibility of a shared pathophysiology.[25,132,179,275] Indeed, this view is supported by previous studies that reported profound underscaling of balance correcting responses in freezers,[326] as well as a defective StartReact effect.[269,349] The present findings, however, strongly argue against the suggestion of a common underlying mechanism. The assessment of balance correcting responses combined with a StartReact paradigm in a carefully balanced group of PD patients enabled us to identify hypometric balance correcting responses being specific to postural instability, versus defective StartReact being specific to freezing. The absence of correlations between SAS-induced accelerations of postural responses and continuous markers of postural instability such as amplitudes of postural responses, step length and leg angles particularly speaks in favor of dissociated mechanisms.

Underscaling of balance responses underlies postural instability

Patients with evident postural instability (HY3) had smaller amplitudes of automatic postural responses and a reduced length of the balance correcting step compared to patients without evident postural instability (HY<3). This resulted in a lower quality of the first balance correcting step, as reflected by more negative leg angles and larger numbers of steps needed to recover from the balance perturbations. These

findings are in line with previous studies that also reported similar underscaling of balance correcting responses (including stepping) in PD patients compared to controls.[23,175,192,326,336] These studies, however, did not differentiate between H&Y stages. Importantly, the present results show that not only patients with evident postural instability, but also those without (clinically-defined) postural instability had smaller balance correcting steps and poorer step quality compared to healthy controls. The significant correlations of hypometric response amplitudes and step lengths with step quality highlight the degree of underscaling being the critical determinant of PD-related balance impairments.
The observation of underscaled balance correcting responses in PD patients without evident postural instability is indicative of a continuum of balance impairments in PD, which calls for more sensitive clinical tests to identify and monitor these impairments.

The precise mechanisms underlying the underscaling of balance correcting responses are not completely clear. Moreover, it is unknown whether the underlying mechanisms are the same for hypometric postural responses and for reduced step lengths. The observation that step length tended to correlate with postural response amplitude may point at a common pathophysiological mechanism. Although automatic postural responses and stepping responses are organized in different neural structures, the cortex and basal ganglia are involved in shaping both to the demands of the task at hand.[176] The underscaling may thus reflect the hypokinesia that characterizes PD,[306] which presumably results from abnormal proprioceptive-motor integration in the supplementary motor cortex.[90,175] This explanation, however, raises the question why dopaminergic medication has only a small[23,62] or no effect[85,191,192] on balance responses, whereas it is able to improve supplementary motor cortex activity.[247] The minor effects of dopaminergic medication on balance impairments could indicate that lesions in non-dopaminergic pathways primarily underlie postural instability in PD. Deficiencies in cholinergic pathways might be considered, as degeneration of cholinergic neurons is associated with falls,[179] and treatment with the acetylcholinesterase inhibitor donepezil reduced the number of falls in PD patients.[71] Moreover, bilateral lesioning of the cholinergic part of the PPN in monkeys induced postural deficits.[179] In humans, postural instability in PD is correlated with both electrophysiological[305] and PET-imaging[33,244] measures of PPN-cholinergic dysfunction. Although deficits in non-dopaminergic pathways seem to be of great importance with regard to balance impairments in PD, the marginal effects of dopaminergic medication do not necessarily preclude a role for dopamine deficiency in the underlying pathophysiology, because the threshold for therapeutic relief may simply be higher than for other symptoms.[142] Hence, future studies should further investigate the role of dopaminergic as well as non-dopaminergic pathways in the underscaling of balance correcting responses.

Disturbed StartReact in freezers

In both PD patients and controls, a smaller SAS-induced acceleration was observed during postural responses compared to the ankle dorsiflexion task, which is in line with the literature.[265,266,268] There is, however, strong evidence that postural responses to balance perturbations are preprogrammed and subject to triggered release by a SAS, resulting in a StartReact effect.[50,51,266] The smaller degree of acceleration by a SAS might be explained by differences in neural organization. In contrast to voluntary reactions in response to an imperative auditory or visual stimulus, automatic postural responses do not involve transcortical pathways,[176,292,348] but are likely encoded by assemblies of neurons in the pmRF.[337] The observation of defective StartReact effects in freezers is novel for automatic postural responses following backward balance perturbations. Previous studies demonstrated similar results for simple ballistic movements of the upper and lower extremities and when initiating gait.[269,349] The consistency of these findings in different tasks suggests a common origin, possibly involving dysfunction of upper brainstem structures.[179,349] Here, we extend on these findings by showing that defective StartReact is specific to freezing of gait and is not related to postural instability.

The occurrence of the StartReact effect critically depends on the upcoming movement being readily prepared and 'stored'. The exact neural structures involved remain to be unraveled,[3] but there is accumulating evidence that StartReact reflects direct release of subcortically stored motor programs, possibly from the pontomedullary reticular formation (pmRF).[271,359] Our group investigated the StartReact effect in patients with hereditary spastic paraplegia (HSP). Patient with HSP have retrograde axonal degeneration of the corticospinal tract,[177,289] while the reticulospinal tract is not affected.[265] In these patients, ankle dorsiflexion reaction times to a visual stimulus were delayed, which finding concurred with delayed corticospinal motor conduction times as measured with supramaximal TMS. Upon the presentation of a visual stimulus combined with a SAS, however, they exhibited similar latencies to healthy control subjects, irrespective of the presence of a SCM-reflex.

Based on this notion of a SAS-induced release of subcortical motor program, the defective StartReact effect in freezers may either indicate poor movement preparation at this levelor reduced responsiveness of these structures to triggers releasing the prepared motor responses. Finally, the attenuated StartReact effect in patients with FOG can be the result of an increased gain of the reticulospinal output.[125] In freezers, the gain of the reticulospinal output might be set at maximum to compensate for underlying degenerative changes. In that case, the gain cannot increase further and the SAS will have no additional effect on a voluntary reaction time task, such as the present ankle dorsiflexion task, nor on corrective postural responses. However, our results on the effects of a SAS on the scaling of responses do not seem to argue in favor of differences in gain underlying the attenuated StartReact effects in freezers.

Stepping leg angles significantly improved when the SAS was applied together with the perturbation, which effect was similar between freezers and non-freezers. Furthermore, in our previous paper on StartReact effects in gait initiation, we reported greater amplitudes for anticipatory postural adjustments as well as greater response amplitudes in the tibialis anterior in trials with a SAS compared to those without.[269] Again, these effects of the SAS on response scaling were not different between freezers and non-freezers, whereas the effects of the SAS on response onsets were similar to those presently reported.

Importantly, there are several observations that suggest that startle reflexes and StartReact effects are at least partly dissociated. First, while a prepulse at 100 ms and 500 ms was shown to significantly reduce the amount of SCM activation, the StartReact effect was reported to be unaffected by the prepulse.[222] Second, the presence of a startle reflex in SCM does not appear to be a prerequisite for the StartReact effect. Although some studies[56,163,353] reported an attenuation of StartReact effects when no startle reflex activity is observed, several other studies could not establish a significant relationship between the occurrence of SCM responses and StartReact, which was true both for experiments involving simple reaction time tasks[219,271] and for tasks involving gait and postural responses.[52,209,266,271,300,307] As a SAS restored reaction times in patients with HSP, irrespective of the presence of a SCM-reflex, startle reflexes are likely not a prerequisite for a SAS-induced release of a subcortically stored motor programs. A final observation in support of (at least partly) dissociated mechanism underlying startle reflexes and StartReact comes from a study that reported on the effect of PPN-stimulation in PD patients with FOG.[349] When the stimulator was turned off, the StartReact effect and startle reflexes were absent in these patients, but PPN-stimulation restored StartReact effects, while leaving the impairment in startle reflexes unchanged. This implies that a defective StartReact effect in freezers does not merely represent a degradation of primary startle reflex pathways, but rather points at a specific motor preparation deficit.

Remarkably, the putative inadequate representation or release of motor programs at brainstem level in freezers did not result in delayed postural responses, despite the fact that these responses are presumably mediated also by neurons in the pmRF.[337,359] Given the great complexity of neuronal organization at the brainstem level, and uncertainties regarding the integrative role of brainstem nuclei in the generation of movement, we can only speculate about a possible explanation for this rather unexpected result. Possibly, the postural response is released from different (brainstem) neurons when triggered by a SAS as compared to the proprioceptive input induced by the mechanical perturbation itself. This implies that some neurons may be primarily involved in the preparation of the automatic postural response, whereas others are responsible for its release by afferent information. By analogy with

voluntary movements, the SAS could have access to neurons that are involved in the preparation of the response.[359] In freezers, these preparatory structures may be defective, whereas the neural circuits involved in the release of automatic postural responses by proprioceptive input may be intact. Further research should identify the existence of such brainstem networks in order to support this hypothesis.

In conclusion, our results suggest that the mechanisms underlying freezing of gait and postural instability in PD patients are at least partly different. This stresses the notion that future studies should address gait and balance separately,[213] and calls for studies elucidating the specific neural circuits subserving these behaviors, as well as their degradation in PD. Novel paradigms, such as motor imagery of balance control and gait,[123] could help to further unravel the separate mechanisms underlying postural instability and FOG.

10

Freezing of gait: a practical approach to management

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Abstract

Freezing of gait (FOG) is a common and disabling symptom in patients presenting with parkinsonism, characterized by sudden and brief episodes of inability to produce effective forward stepping. These episodes typically occur during gait initiation or turning. Treatment is important because FOG is a major risk factor for falls in parkinsonism, and a source of disability to patients. Various treatment approaches exist, including pharmacological and surgical options, as well as physiotherapy and occupational therapy, but evidence is inconclusive for many approaches, and clear treatment protocols are not available. To address this gap, we review medical and non-medical treatment strategies for FOG and present a practical algorithm for the management of this disorder, based on a combination of evidence, when available, and clinical experience of the authors. Further research must formally establish the merits of our proposed treatment protocol.

Introduction

Freezing of gait (FOG) is a common and incapacitating symptom that occurs in patients with Parkinson's disease (PD), and even more frequently in most forms of atypical parkinsonism. Additionally, FOG can occur in isolation in patients with primary progressive freezing of gait; this disorder is often a prelude to later development of progressive supranuclear palsy (PSP) or another tauopathy. In PD, FOG is associated with disease severity,[208] although it can be seen early in the course of the disease. However, if FOG is (one of) the first presenting signs, atypical forms of parkinsonism should be suspected.[132] Freezing is not restricted to gait, and can also occur in alternating repetitive movements of the fingers[6,259] and during speech.[236] Whether these other motor blocks have the same pathophysiological substrate as FOG is unclear.

FOG is characterized clinically by sudden, fairly brief episodes of inability to produce effective forward stepping that typically occur during gait initiation or turning while walking.[275,276] These gait blocks greatly interfere with daily life. Importantly, FOG is now recognized as one of the main risk factors for falls (because during walking, the trunk keeps moving while the feet become stuck).[53] This risk is compounded by the fact that FOG often co-occurs with substantial balance problems[25] and cognitive (mainly frontal executive) deficits.[134]

Treatment of FOG is perceived by clinicians as a very challenging task. The need for a treatment protocol with a clear decision algorithm is widely acknowledged, but such a protocol does not exist.

Here, we provide an overview of the medical and non-medical management of FOG, including use of drugs and surgical approaches, non-pharmacological therapies, and treatment of co-morbidities. We first discuss the need for careful history taking and clinical assessment to accurately diagnose FOG and to assess its (subjective) severity; we then present an algorithm for the practical management of FOG. All recommended interventions are based on evidence when available (classified according to their level of evidence in Table 1 and 2). Otherwise, our recommendations reflect practice-based evidence supported by our clinical experience.

History taking and provocation of FOG

Several papers provide a detailed description of both history taking and clinical provocation of FOG;[328,332] here, we provide a brief summary. Simply asking the patient whether freezing has occurred is usually insufficient to identify whether or not FOG is present. Instead, we recommend asking whether the patient has ever experienced the characteristic feeling of the feet being glued or pasted to the floor, or being stuck to the floor, as if attracted by an invisible magnet. To ascertain that the patient really understands what FOG is, it can help for the examiner to stand up and imitate a FOG

Table 1 Classification of level of evidence.

A1	Meta-analysis containing at least some trials of level A2 and of which the results of the trials are consistent.
A2	Randomized comparative clinical trials of good quality (randomized double-blind controlled trials) of sufficient size and consistency.
B	Randomized clinical trials of moderate (weak) quality of insufficient size or other comparative trials (non-randomized, cohort studies, patient-control studies
C	Non-comparative trials
D	Expert opinion

episode or, preferably, to show a video of a typical FOG episode in a patient. Investigation of circumstances during which FOG occurs is necessary (e.g. during turning, or under time constraints). Moreover, frequency, intensity, and duration of FOG should be discussed. The New-Freezing of Gait Questionnaire can be helpful to assess the subjective severity of FOG and effects on daily life.[258] Additionally, the subjective effect of (dopaminergic) medication should be assessed, by asking whether freezing occurs predominantly, or even exclusively, when the medication has worn off (the so-called off-state), or whether FOG occurs both in both the 'OFF' and 'ON'-state (characterised by improvement of other symptoms compared with the off-state). Answers to these questions usually provide an accurate portrayal of the treatment response to dopaminergic medication. Finally, asking about the presence of falls can be helpful, since FOG episodes are recognized as a major cause of falls in PD.[53] Fall types typically related to FOG include falling while turning, and apparently spontaneous falls (often the patient has missed a brief FOG episode that preceded the fall).

FOG is an unusual gait disorder because of its episodic character. Provocation of FOG during neurological examination is therefore difficult. The patient's extra attention to gait during clinical examination can probably temporarily suppress freezing. Additionally, FOG is less likely to occur in a widely spaced hospital corridor, which is unlike the sometimes tight quarters in the patient's own living space. To provoke FOG, asking the patient to make full and rapid turns in both directions,[332] or to walk with short steps as rapidly as possible can help.[270]

The initial treatment of mild FOG

The first step in our treatment algorithm (Figure 1) is to decide whether or not FOG is troublesome to the patient. Troublesome is operationally defined here as interfering with the patient's mobility or quality of life, for example when FOG is associated with social embarrassment or fear of falling, or actually leads to (near) falls. For some patients, and certainly in early stages of development, FOG can be mild and does not

Table 2 Level of evidence of interventions for FOG.

Type of FOG	Intervention	Effect	Level of evidence
All types of FOG	Physiotherapy • rhythmic auditory cues and visual cues • walker or stick projecting a laser line on the floor • psycho-education	 Improvement Improvement Improvement	 Level B[186,187,242,257] Level C[101] Level D
	Occupational therapy • home adjustment • help with daily planning	Improvement	Level D[344]
Dopamine-responsive FOG	Levodopa	Improvement	Level B[113,114,313]
	Dopamine-agonist	More new FOG episodes compared with levodopa Effect of dopamine-agonist vs placebo on FOG has not been investigated. Expert opinion: dopamine-agonists can both worsen and improve FOG.	Level A2*[295] Level D
	Monoamine oxidase type-B inhibitors (rasagiline or selegiline)	Reduced risk of developing FOG	Level A2*[132,296]
	STN-stimulation	Improvement	Level C[304,365]
	GPi-stimulation	Effects on FOG needs to be investigated	-
	Methylphenidate	Improvement in patients after STN-stimulation, but no improvement in general	Level B[238] Level B[111]
	Intraduodenal levodopa gel	Improvement	Level C[91]
	Apomorphine	Effect on FOG needs to be investigated	Level D
	Amantadine (either orally or intravenous)	Inconsistent data	Level C[133,203,208,216]
	Electroconvulsive therapy	Insufficient data	Level D[286]
	Transcranial direct current stimulation	Insufficient data	Level D[354]

Table 2 Continued.

Type of FOG	Intervention	Effect	Level of evidence
	PPN-stimulation	Inconsistent data	Level C[122,240,350]
	Botulinum toxin injections	No improvement	Level B[120,146,377]
Dopamine-resistant FOG	Droxidopa plus entacapone	Insufficient data	Level C[129]
	Intraduodenal levodopa gel	Improvement	Level D[65]
	STN-stimulation	No improvement	Level C[40,66]
	Amantadine	No improvement	Level B[190]
Dopamine-induced FOG	Reduction levodopa	Improvement	Level D[112]
	STN-stimulation	Improvement	Level C[121]

*FOG was not the primary outcome measure, but a secondary or fortuitous endpoint

yet interfere with daily function. Importantly, even mild symptoms of FOG need to be taken seriously, because mild FOG almost inevitably progresses to troublesome FOG. Therefore, regular assessment of its impact on the patient is needed.

Troublesome FOG should always be treated aggressively. However, all patients, including those with mild FOG, should be educated about FOG, especially about the risk of falls, various provoking circumstances and possible preventive measures (Table 3). In patients with mild FOG, we therefore always recommend physiotherapy. Physiotherapy includes both dedicated strategies (cues) that can assist patients to overcome the FOG episodes (e.g., conscious movement strategies to increase step amplitude, retaining stepping rhythm, making lateral weight shifts, directing attention to gait and making wide arcs when turning) and the recommendation to maintain sufficient exercise levels.[242,257] Although no evidence exists that exercise can prevent or decrease FOG, stimulation of physical activity in PD patients is generally regarded to be important. Cycling can be advised, at least in countries where outdoor cycling is very prevalent, such as The Netherlands or Japan, because patients rarely experience FOG during cycling.[233,330] A tricycle or a stationary bicycle at home can be considered for patients who are not used to cycling (eg, in countries where outdoor cycling is not part of the culture), or who have difficulty mounting or dismounting owing to balance problems.

Several additional measures can be considered for patients with mild FOG. One consideration is to prescribe MAO-B inhibitors, such as rasagiline and selegiline, because clinical trials have shown that these are associated with reduced risk of future FOG (for both drug-naïve patients and patients already receiving other dopaminergic

drugs).[132,296] FOG was not the primary outcome in these clinical trials, so this finding might have been incidental; future studies with FOG as a primary outcome are needed to further validate this strategy. This strategy aims to prevent possible development of FOG, and not to symptomatically treat overt FOG. We have tried symptomatic treatment of freezing of gait, with very limited success.

The approach to troublesome FOG

For patients with troublesome FOG, management consists of three pillars: medical treatment (drugs and deep brain stimulation); non-pharmacological therapies; and assessment and treatment of co-morbidities.

Pharmacological and surgical treatment options

The first crucial step in the approach of troublesome FOG is a detailed assessment of when freezing occurs with respect to medication dosages. In this assessment, identification of which of three types of FOG is present is important: dopamine-responsive (i.e. related to loss of central dopamine); dopamine-induced (i.e. caused by the administration of dopaminergic medication); or dopamine-resistant (i.e. related to presence of non-dopaminergic brain lesions). A detailed diagnostic approach to differentiate between these types of FOG has been presented;[112] we give a summary here, with recommended therapeutic strategies.

Dopamine-responsive FOG

We recommend that FOG should always be approached first as being dopamine-responsive, because this is the most common phenotype of FOG, especially in early disease stages of PD.[112,275] Indeed, many patients first experience FOG exclusively when medication wears off. A careful assessment of this possibility and management of early response fluctuations (ie, the patient's variable response to dopaminergic medication, as reflected by transitions between a patients' relatively good motor performance when the dopaminergic medication Is effective and a more prominent parkinsonian state when the dopaminergic medication has transiently lost its effect) often alleviates or even resolves the problem, at least initially. A typical feature is that episodes of FOG are more common and more prolonged during the 'OFF' state than during the 'ON' state.[313] Adequate treatment of dopamine-responsive FOG seems to need higher doses of dopaminergic medication than does suppression of other cardinal signs of PD, such as bradykinesia and rigidity.[112] This means that one can encounter patients whose upper body signs – and in particular the fine hand movements – seem to be in an ON state, while the legs continue to manifest FOG. A tempting conclusion would be that such patients have ON state FOG, but in our experience many patients have improved walking when increased levodopa doses are tried, sometimes at the expense of upper body dyskinesias.

Figure 1 Algorithm for the practical management of FOG.

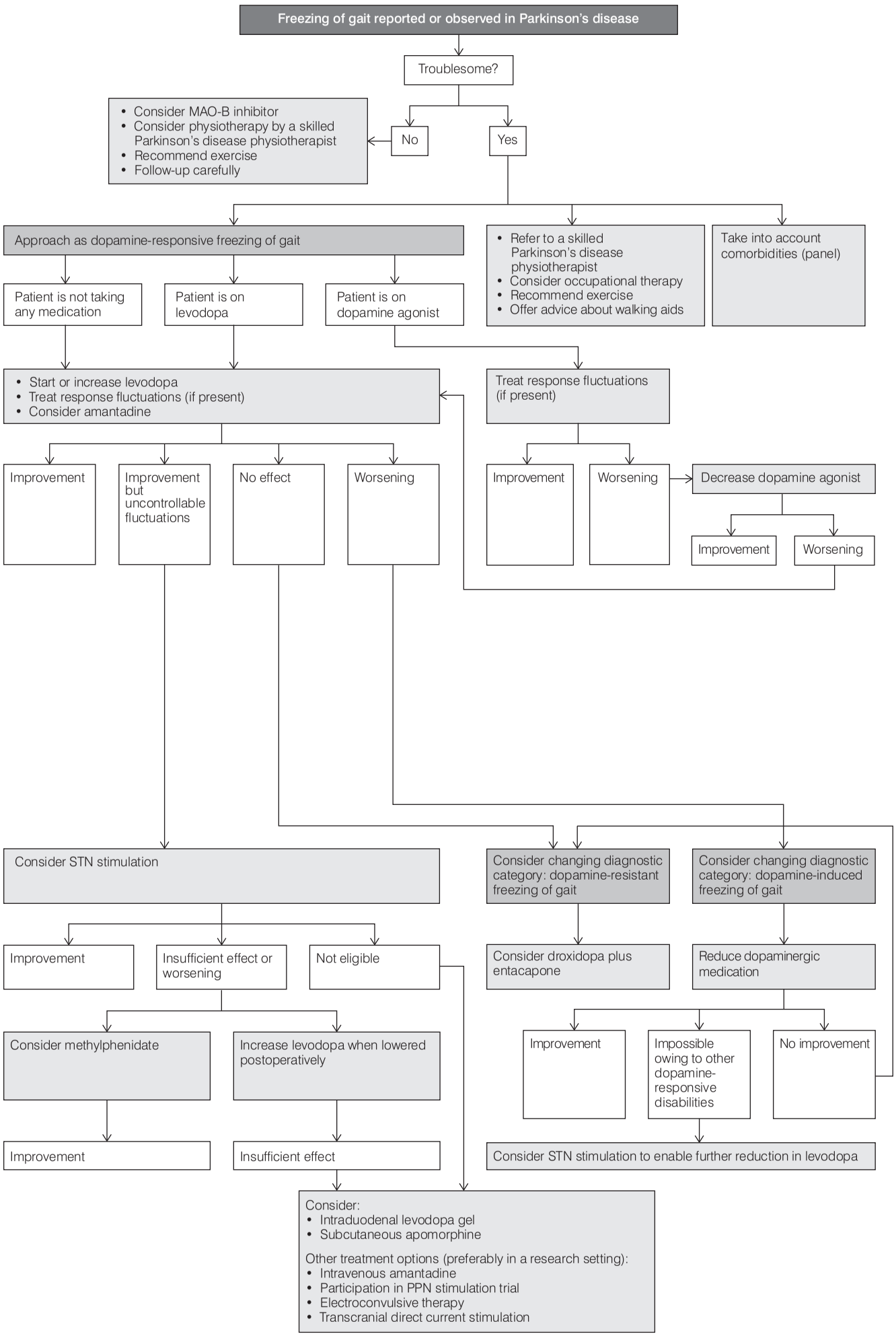


Table 3 Provoking circumstances and preventive measures in relation to FOG.

Provoking circumstances	Preventive measures
Gait initiation	Shifting weight to one leg before swinging the other leg forward
Narrow turns	Take a wide turn, stepp over a line or companion's foot
Tight quarters	Create wider spaces (home visit by occupational therapist)
Time pressure	Behavioural modification
Crowded situations	Anxiety control
Dual tasking	Focus attention to gait

The preventive measures listed in this table are based on experts' opinion.

The first step in the drug treatment of FOG is to assess the effect of levodopa (in treatment naïve patients), or to increase the dose in patients already treated with levodopa, to at least 1000 mg/day if needed. If FOG only partly responds to such high doses of levodopa, and if the patient is not hindered by dose-limiting adverse effects, then dosage can potentially be increased further to improve control of FOG. If FOG occurs mostly when the medication has worn off (which is often the case), patients can benefit from the well-known strategies to alleviate response fluctuations (eg, reduction of time intervals between the subsequent medication intakes).[74] Patients who experience FOG when getting up at night to go to the bathroom might benefit from controlled-release preparations before sleeping to reduce night-time OFF periods. Levodopa is our first choice in treatment-naïve patients, because an incidental observation in a clinical trial suggests that dopamine agonists are associated with a greater risk of developing FOG than is levodopa.[295] Specifically, FOG was more common in the agonist group than in the levodopa group, but this finding must be interpreted with caution because FOG was not the primary outcome in this trial. Moreover, dopamine agonists are more weakly effective than levodopa, so patients with freezing who were given an agonist might have simply been undertreated. However, in our experience, agonists (irrespective of which) occasionally worsen or even induce FOG, which then disappears when the agonist is discontinued and does not return when levodopa is subsequently introduced (see below). When we encounter a patient who has developed FOG while being treated with a dopamine agonist (either as monotherapy or as part of polypharmacy), we first assess whether FOG occurs mostly when the medication has worn off. A first step should be to treat the response fluctuations, which could include increasing the dose of the dopamine agonist if the dose is fairly low (note that patients with so-called agonist-induced FOG usually do not have response fluctuations). However, we would be inclined to recommend not increasing the dopamine agonist as the first step, but rather to start levodopa or

increase the levodopa dose or one of the other established strategies to treat response fluctuations. If FOG worsens despite successful reduction of response fluctuations, we assess the effect of reduction of the dopamine agonist and perhaps stop the agonist altogether.

In addition to dopaminergic medication, we consider oral administration of amantadine in patients with dopamine-responsive FOG, although the supporting evidence is inconclusive [133,208,216] and further studies are needed. We recommend a trial of amantadine, undertaken judiciously (100 mg per day) in view of its common side-effects, especially in elderly patients. However, if tolerated, amantadine doses as high as 600 mg total per day could be used, if no dose-limiting side-effects occur.
Levodopa treatment is often complicated by dose-limiting side-effects, resulting in suboptimal treatment of dopamine-responsive FOG. In such cases, deep brain stimulation of the subthalamic nucleus (STN) can be considered, especially when several reasons exist to move towards surgery. Studies on the effect of STN-stimulation on dopamine-responsive FOG are fairly small (the largest number of patients to be treated with STN-stimulation is 20)[365] and have a fairly brief follow-up (on average one year post-surgery). The limited evidence suggests that STN-stimulation can reduce the occurrence of dopamine-responsive FOG, as measured with freezing of gait questionnaires.[304,365] Additionally, several studies have reported beneficial effects of STN-stimulation on spatiotemporal gait characteristics, and improvement in UPDRS scores of postural instability and gait disability (UPDRS-PIGD-scores).[13] STN-stimulation and dopaminergic therapy combined can result in a further improvement of UPDRS-PIGD-scores and spatiotemporal gait characteristics, as compared with STN-stimulation alone.[115,207,340,381]

No studies have directly investigated the effect of deep brain stimulation of the internal globus pallidus (GPi) on the occurrence of dopamine-responsive FOG. The effect of GPi-stimulation on spatiotemporal gait characteristics has been investigated,[4,87,287] with beneficial effects on step length, but inconclusive effects on other parameters such as cadence, velocity and double support time (the time during which both feet are on the ground). Additionally, beneficial effects on UPDRS-PIGD-scores have been reported.[13] Because the effect of GPi stimulation on dopamine-responsive FOG occurrence has not been formally documented, we prefer STN-stimulation to GPi-stimulation. However, future work is needed, because GPi-stimulation has been suggested to offer an improved long-term perspective for gait and balance deficits compared with STN-stimulation.[126,241,335] These future studies should closely monitor for potential worsening of FOG after GPi-stimulation, because stimulation-induced FOG has been reported as an adverse effect of GPi-stimulation in patients with dystonia.[319]

Some patients develop or have worsened FOG and other axial motor problems several years after deep brain electrodes have been implanted,[121,197,361] possibly as a result of natural disease progression. In such patients, we recommend increasing levodopa dosage, because dopaminergic medication is typically lowered substantially after implantation of STN electrodes (thereby effectively unmasking dopamine-responsive FOG). Another option is to adjust the stimulator settings[118]; beneficial effects have been described when stimulation frequency is decreased to as low as 60 Hz,[237,302] when stimulation voltage is lowered[9,117,382] or when left-right asymmetry in stimulator settings is minimised.[116] In our experience, adjustment of stimulator amplitude should be the first step, and sufficient time after the adjustment should be allowed to fully judge the effect; adjustment of the frequency or symmetry of stimulation are secondary steps. If a patient with optimally tuned STN-stimulation (plus adequate levodopa treatment) continues to manifest FOG, we recommend a judicious trial of methylphenidate (1 mg/kg per day), because beneficial effects have been reported in this specific subgroup of patients.[238] Effects of methylphenidate for patients with FOG who have not undergone surgery are not convincing.[111] Further large scale randomised clinical trials are needed to understand the role of methylphenidate in advanced PD with troublesome FOG, and to examine its working mechanism (eg, direct effect on FOG, or possibly an indirect effect via increased alertness).

When deep brain stimulation is not appropriate, a few dopaminergic treatment options can be considered for which no meta-analyses or randomised double-blind controlled trials exist (Table 2). Intraduodenal levodopa gel and subcutaneous apomorphine infusions provide more continuous dopaminergic stimulation and are associated with fewer motor fluctuations compared to oral levodopa.[153,379] For suitable patient subgroups (those with severe response fluctuations that cannot be controlled with oral medication, and with contra-indications for deep brain stimulation), intraduodenal levodopa gel and subcutaneous apomorphine injections or continuous infusion can be considered for the management of FOG. Although positive effects can be expected in such patients, only one (fairly small) study[91] has assessed formally the effect of intraduodenal levodopa gel on gait disorders, including freezing of gait, festination and postural instability. In this retrospective study, clinicians were asked to rate the effect of intraduodenal levodopa infusion on a three-point scale: improvement, no change or worsening. Gait improved in 46 out of 75 PD patients (61.4%) treated with intraduodenal levodopa infusion, whereas it did not change in 28 patients and worsened in one patient.[91] For subcutaneous apomorphine infusions, no studies have been done to our knowledge. We therefore recommend preferential use of these therapies in a research setting, but patients who are good candidates (ie, those with a contraindication for deep brain stimulation, or patients who prefer either intraduodenal

levodopa gel or apomorphine) can also receive these treatments in daily clinical practice. Amantadine given intravenously seemed to improve secondary outcomes in a single study, but this remains an experimental therapy that should first be studied in more detail.[203] Amantadine could work through the dopaminergic system, but improvement through its known effect on fatigue or on alertness cannot be excluded. In view of the high bioavailability of oral amantadine, the reason for intravenous treatment being superior is not clear, and placebo effects cannot be excluded; this is another area that should be investigated in controlled trials.

(Partially) dopamine-resistant FOG

With disease progression and increased disease duration, partial dopamine resistance develops in most patients with initially dopamine-responsive FOG, partly because dose-limiting response fluctuations make delivery of adequate doses increasingly difficult. Additionally, this dopamine resistance can partly be ascribed to progressive development of non-dopaminergic brain lesions involving, for example, the frontal lobe, adrenergic locus coeruleus[140] or the cholinergic portion of the pedunculopontine nucleus (PPN).[238] However, FOG that is completely dopamine resistant is uncommon,[112] and we suggest that clinicians should follow the algorithm depicted (Figure 1) before reaching this conclusion.

Non-dopaminergic drugs could potentially reduce FOG occurrence, both for partially and totally dopamine-resistant FOG, but, so far, results have been disappointing, and no meta-analyses or randomised double-blind controlled trials exists. In our opinion, these treatment options should be reserved for research settings. Non-dopaminergic treatment options have been investigated mostly in patients who initially presented with dopamine-responsive FOG, and are therefore listed under that category in Table 2. One approach focuses on correcting deficits in adrenergic circuitries, including treatment with the combination of droxidopa and entacapone. This approach is listed under the category dopamine-resistant FOG, because beneficial effects on FOG were reported in this group.[129] However, whether the patients in this study were completely, partly, or even apparently resistant to levodopa is not clear. Another approach focuses on correcting deficits in cholinergic pathways, which seem to contribute to dopamine-resistant FOG.[34] Central cholinesterase inhibitors reduce falls in PD patients with postural instability,[71] which is potentially interesting because FOG is so closely related to falls. However, most patients included in this trial did not experience FOG, and fall rates did not improve in those with FOG. Future studies are needed to investigate the effect of cholinesterase-inhibitors on the occurrence of FOG. On the basis of our clinical experience, we would not expect striking effects, because patients who receive cholinesterase-inhibitors (with the aim of improved cognition) rarely have substantial improvements in FOG.

Patients whose FOG does not respond to dopaminergic therapy will neither improve with deep brain surgery targeted at either the STN or GPi. Deep brain stimulation of the PPN is one of the non-dopaminergic treatment options that can be considered. However, experience with PPN-stimulation is inconsistent,[122,240,350] with improved scores on FOG-questionnaires in one study,[350] but no overall improvement of questionnaires in another study.[122] The optimum stimulation target within the large, diffuse PPN remains to be investigated, and whether the PPN proper needs to be targeted or whether the cuneiform or subcuneiform nuclei need to be targeted is unknown.[2] Moreover, work is needed to define the best possible treatment candidates. Until such evidence becomes available, PPN-stimulation is an experimental procedure that, in our opinion, should be studied only in a research setting.

Improvement FOG questionnaire scores and reduced fall frequency were reported in a study of five patients with dopamine-resistant FOG after treatment with 24-hour levodopa-carbidopa intestinal gel (with the night-time rate at 50-80% of the daytime infusion rate).[65] The underlying mechanism needs to be investigated, but might be related to improved sleep quality, resulting in subsequent improved daytime motor performance. A large prospective placebo controlled study is needed to verify these observations.

Non-invasive brain stimulation techniques might reduce occurrence of FOG in patients with partially dopamine-resistant FOG. Both electroconvulsive therapy (ECT)[286] and transcranial direct current stimulation (tDCS)[354] reduced the number of FOG episodes in small studies. The underlying mechanism is not known, but might be non-dopaminergic. Dopaminergic mechanisms might also be involved, because ECT has been suggested to enhance sensitivity of postsynaptic dopaminergic receptors,[286] and tDCS can induce dopamine release in the basal ganglia.[354]

Treatment with botulinum toxin into calf muscles has been tried to alleviate FOG. However, results of several studies have shown that this approach does not to improve FOG,[120,146,377] and this treatment option is therefore discouraged.

Dopamine-induced FOG

FOG can occasionally be caused by dopaminergic medication.[112] No properly prevalence studies have been done, but in our experience, dopamine-induced FOG is rare (presumably less than 5% of cases). Patients with true dopamine-induced FOG generally report walking better at night (when medication has worn off) than during the day – eg, when visiting the toilet because of nocturia, or early in the morning (before taking their first daily dose of dopaminergic medication). Improved

gait during the night or in the early morning could be a sleep benefit effect,[360] although recent work by our group suggests that sleep benefit is largely subjective and does not translate into objective motor improvement. In patients with true dopamine-induced FOG, well-intended attempts to improve gait with increasing doses of dopaminergic medication only worsen FOG. The mechanism underlying dopamine-induced FOG is unknown. Dopamine-induced FOG can occur in patients treated with levodopa,[112] but as noted above, we have also seen clear FOG induced by agonist monotherapy (which disappeared when the agonist was stopped, and did not recur when levodopa was given). This finding suggests a complex interaction between medication and various types of dopamine receptors in the pathophysiology of FOG. Hypothetically, dopaminergic medication might worsen FOG indirectly via its influence on cognitive performance, and particularly via a negative effect on frontal executive functions and alertness; this hypothesis needs to be investigated in future studies. In low doses, dopaminergic medication can improve cognition, but increasing doses can negatively affect cognitive functioning (U-shaped curve).[75] Executive functions might thus deteriorate in some patients when the dosage of dopaminergic medication is increased, resulting in development or worsening of FOG (eg, because of disturbed motor planning or impaired attention). Monitoring of cognitive functions and alertness in patients with FOG is crucial, both ON and OFF medication.

Dopamine-induced FOG is treated mostly by reducing dopaminergic medication. The agonist should be reduced first, followed by levodopa. Switching to another agonist has not been reported to be successful. When the necessary reduction of dopaminergic medication is impossible owing to unacceptable worsening of other PD-related dopamine-responsive signs, such as severe tremor or rigidity, we consider STN-stimulation to be a last-resort treatment for dopamine-induced FOG. This intervention does not act directly on dopamine-induced FOG, but only alleviates the problems indirectly by enabling a substantial reduction in the postoperative dosage of dopaminergic medication.

Non-pharmacological therapy

Non-pharmacological therapy includes the same physiotherapy strategies discussed for mild FOG. Additionally, knowing that FOG is influenced by constraints in the physical environment, we recommend involving an occupational therapist who can advise about possible domestic adaptations, such as removal of obstacles, optimising of light, or provision of safety rails.[344] Moreover, FOG can increase during stressful situations, and occupational therapists can assist with planning of daily schedules, aiming to minimize stressful moments. Together with a physiotherapist, occupational therapists can offer advice about assistive walking aids such as light folding wheelchairs. Walking aids can be useful, but can paradoxically worsen FOG

in some patients, so training the patient in use of the walking aid is important. When a walker is necessary, a wheeled walker is preferred.[79] Patients who respond to visual cues may benefit from a walker or stick projecting a laser line on the floor to step over.[101] Guidelines recommend that physiotherapists and occupations therapists should give short consultations, aiming to educate patients and thereby support their independence, rather than offering long-term treatment.[188,342] However, both disciplines should remain available for renewed consultation as the disease progresses and potential new problems arise. When prescribing physiotherapy or occupational therapy, we recommend referral to professionals who have received specific training in use of these PD-specific strategies, and who have a high caseload of patients with parkinsonism.[27,245,343] Unfortunately, access to physiotherapy and occupational therapy (and particularly to skilled therapists with dedicated expertise in delivery of cueing strategies, for instance) is not available in all countries.

Co-morbidity

The presence of various comorbid disorders (Panel 1) can negatively affect FOG, and these should be treated when possible. As mentioned before, treatment of cognition with cholinesterase inhibitors rarely has a strong beneficial effect on FOG. In our experience, depression and anxiety are better treatment targets than cognition. Anxiety is common in patients with FOG, both as a trigger for FOG events (eg, in crowded places, or during time-constrained situations) and as a result of FOG (including a fear of falling). Occupational therapists can assist with planning of daily schedules, aiming to minimize stressful provocative events. If this approach seems to be unsuccessful, or if anxiety strongly interferes with daily life activities, it is our experience that an anxiety-lowering strategy offered by a psychologist can be helpful in reduction of FOG. Additionally, selective serotonin reuptake inhibitors can decrease FOG in some patients, especially in those with comorbid anxiety. However, well-designed clinical trials have not been done. Interestingly, improvement of FOG has been reported in a single patient who received duloxetine – a serotonin and norepinephrine reuptake inhibitor – as treatment for his depression,[239] either indirectly (due to an antidepressant effect) or directly (suppression of FOG via an effect on non-dopaminergic neurotransmitter systems). Replication of this finding in increased numbers and, eventually, in controlled clinical trials remains needed.

Opthalmologic disorders are common in PD. These disorders can be caused by the neurodegenerative process underlying PD, be a comorbid feature of older age, or be a a side-effect of PD-related pharmaceutical and surgical treatment.[11] The combination of FOG with disturbed vision or oculomotor deficits can potentially have a detrimental effect on mobility and increase the risk of falls, especially because many PD patients depend on visually guided movements to compensate for disturbed automaticity in defective basal ganglia circuitries. Disturbed vision and oculomotor deficits are

Panel 1 Co-morbidity negatively influencing mobility and falls in FOG.

Depression (consider treatment with SSRIs or SNRIs)
Anxiety (consider consultation of psychologist or treatment with SSRIs or SNRIs) Disturbed vision
Cognitive dysfunction, executive dysfunction, or both
Orthostatic hypotension Orthopaedic or muscle problems

Treatment recommendations are based on the author's experience. SSRIs=selective serotonin reuptake inhibitors. SNRIs=serotonin-norepinephrine reuptake inhibitors.

therefore potentially important treatment targets, and referral to an optometrist or ophthalmologist should be considered for these patients.

Orthostatic hypotension negatively affects mobility and contributes to falls in patients with FOG, and is therefore an important treatment target. Several treatment strategies are available (not discussed here).[26] Assessment of whether orthostatic hypotension is a side-effect of medication (such as antihypertensive drugs) is important.

Finally, orthopaedic comorbidity (including traumatic lesions related to falling) can further affect gait and balance – eg, by increasing gait asymmetry, which leads to worsened FOG. Alertness to underlying orthopaedic problems and tailored interventions can reduce FOG and improve general mobility.

Freezing of gait in atypical parkinsonism

Owing to a paucity of well-designed clinical trials, the extent to which FOG in patients with atypical parkinsonism improves with dopaminergic medication is unclear. Our experience suggests that a trial of adequately dosed levodopa is justified. High doses of levodopa are often needed to achieve some benefit. Additionally, amantadine could be considered in patients with PSP, because improved scores on FOG-questionnaires have been reported after treatment with amantadine.[196] A placebo-controlled trial in patients with multiple system atrophy showed no effect of amantadine on UPDRS-III gait subscores, but this study did not focus specifically on FOG.[376] Whether amantadine is effective for patients with other forms of atypical parkinsonism remains unknown. Another option for patients with PSP is amitriptyline, which has been reported sometimes to cause substantial mobility improvements in patients treated with this drug for concurrent depression.[109,254] The reports did not state whether or not FOG was also improved, and large controlled studies are needed to confirm these findings. In our experience, amitriptyline often does not improve FOG in patients with PSP. Transient improvement of FOG in a patient with PSP treated by droxidopa has

been reported,[383] but no large clinical trials have been done. Finally, deep brain stimulation is not usually considered an option for patients with atypical parkinsonism, who are generally not good candidates for the procedure because symptoms generally do not improve after deep brain stimulation.[324]

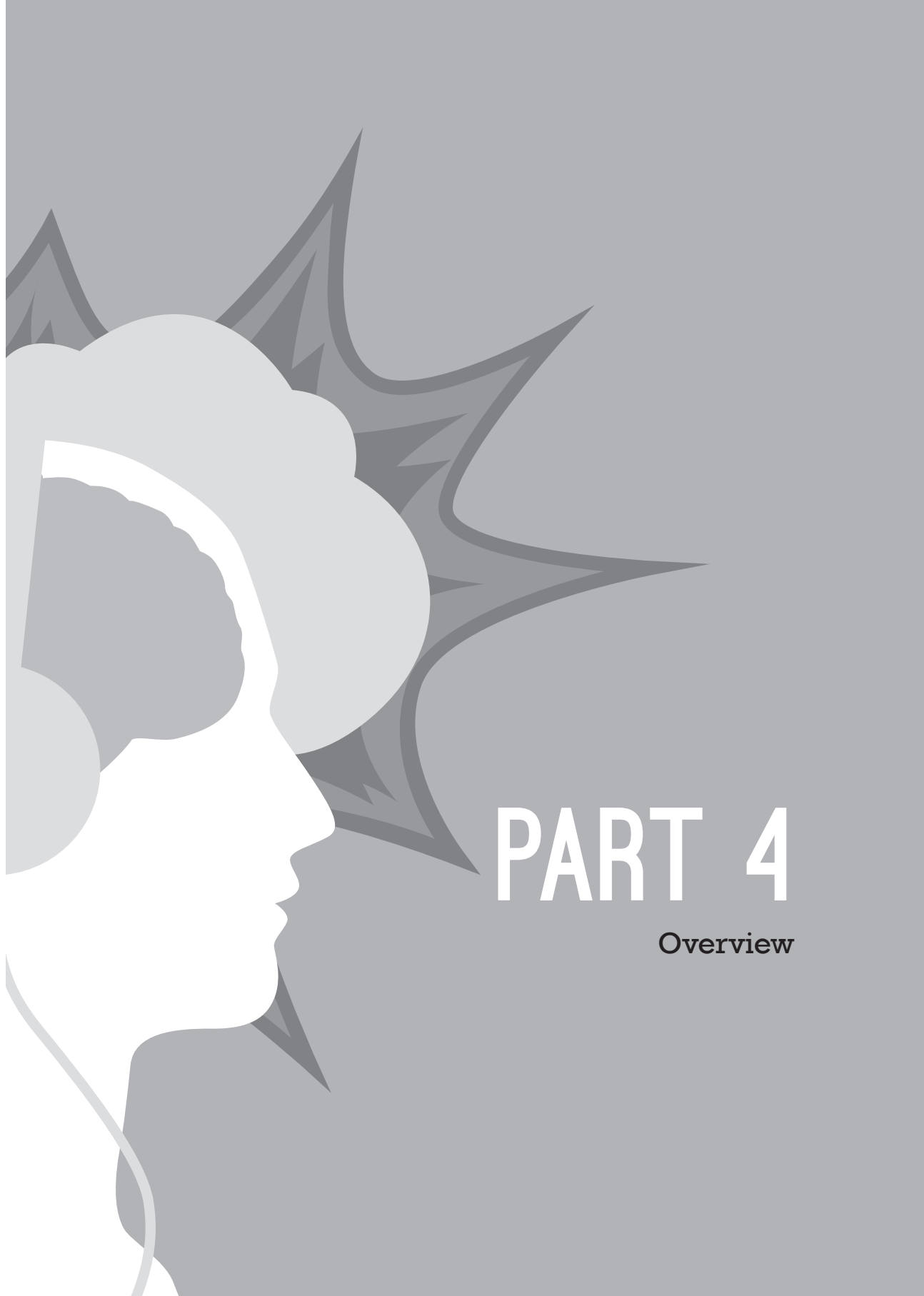
Conclusion

We hope that, pending further evidence, this practical algorithm will support clinicians in their management of FOG in daily clinical practice. However, the level of evidence underlying several steps in our treatment algorithm is currently limited, and further investigation is needed. Randomized clinical trials are needed that include FOG not just as one of many outcomes, but rather as the primary outcome. These future studies should include patients with dopamine-responsive, dopamine-induced and dopamine-resistant FOG, on the basis of unequivocal therapeutic responses obtained during history taking and – if needed – on the basis of observation of FOG before and after a challenge with a supramaximal levodopa dose before inclusion.[112,141] We recommend inclusion of patients whose FOG has been confirmed during neurological examination by an experienced observer (the so-called 'definite freezers').[214] Future studies should use a combination of both subjective assessment (using the validated FOG questionnaire)[258] and neurological examination (which should always include an assessment of rapid turning in place).[332,334] However, even this combination of tests might miss relevant FOG episodes in the patient's own home environment, highlighting the need for development of new measures that quantify the overall amount of FOG across the day. An interesting development is the introduction of wearable sensors (accelerometers or goniometers)[235,374] and perhaps even ambulatory electromyography[73] that might enable objective, continuous and quantitative detection of FOG during daily life. Although the initial findings with use of such sensors is promising,[212] their sensitivity and specificity are imperfect. Further work is therefore needed to identify which type of sensor, which number of sensors, and which positions give the best diagnostic yield for use in future clinical trials. The inadequate evidence base for most available treatments listed in Table 2 suggests a template for the research agenda for this speciality. Development and assessment of new, more effective therapeutic approaches is needed, including pharmacological approaches (in particular non-dopaminergic drugs) and non-pharmacological approaches (such as visual cues provided by smart-glasses). Further investigation of the effect of amantadine on dopamine-responsive FOG and study of the effect of methylphenidate on dopamine-resistant freezing might be worthwhile. Surgical interventions for PD patients are developing at a rapid pace, with beneficial, and sometimes adverse effects on gait;[118,121] the challenge is to identify which targets and

which stimulation protocols offer the greatest improvements in FOG for the different subtypes of FOG. In the speciality of physiotherapy, an interesting challenge is to ascertain whether cueing can be delivered safely and effectively in an on-demand manner –ie, with external cues being delivered only at a time when they are needed most. This challenge depends on development of reliable measures of FOG during free walking and, especially, of early markers that signal the nearby development of a new FOG episode. Initial research in this speciality is promising,[212] but more work is needed. Finally, assessment is needed of whether occupational therapy interventions can help to alleviate FOG.

Search strategy and selection criteria:

We searched PubMed for relevant articles published in English. Searches did not have date restrictions and we included articles up to April 2015. Potential papers were identified using the terms 'freezing of gait', 'Parkinson's', 'parkinsonism' and 'treatment'. Selected articles were also obtained from the reference lists of papers identified by the PubMed search and from searches of the authors' own files. Relevant studied were classified on level of evidence; studies with the highest level of evidence are reported for each treatment option.



PART 4

Overview

11

What startles tell us about control of posture and gait

Published as:
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Abstract

Recently, there has been an increase in studies evaluating startle reflexes and StartReact, many in tasks involving postural control and gait. These studies have provided important new insights. First, several experiments indicate a superimposition of startle reflex activity on the postural response during unexpected balance perturbations. Overlap in the expression of startle reflexes and postural responses emphasizes the possibility of, at least partly, a common substrate for these two types of behavior. Second, it is recognized that the range of behaviors, susceptible to StartReact, has expanded considerably. Originally this work was concentrated on simple voluntary ballistic movements, but gait initiation, online step adjustments and postural responses can be initiated earlier by a startling stimulus as well, indicating advanced motor preparation of posture and gait. Third, recent experiments on StartReact using TMS and patients with corticospinal lesions suggest that this motor preparation involves a close interaction between cortical and subcortical structures. In this review, we provide a comprehensive overview on startle reflexes, StartReact, and their interaction with posture and gait.

Introduction

All of us have experienced the startling sensation of unexpected stimuli. Startling stimuli can result in a startle reflex, which is the fastest generalized motor reaction in humans and animals.[359] Startling stimuli also have the ability to accelerate motor responses, a phenomenon termed StartReact.[55,356] Recently, there has been an increase in the number of studies evaluating startle reflexes and StartReact, many in tasks involving postural control and gait. These studies have helped to improve our understanding on the neural mechanisms underlying startle reflexes and StartReact, and also provided insight into the neural control of posture and gait. Here, a comprehensive review on startle reflexes, the StartReact effect, and their interaction with posture and gait is provided.

Startle reflexes

Neural pathways and characteristics of the startle reflex

A startle reflex is an involuntary motor reaction to unexpected sensory input and consists of a generalized flexion response that follows a rostro-caudal progression.[41,69,378] Startle reflex activity is most prominently seen in the sternocleidomastoid muscle. Subsequently, the descending volley may activate more distal muscles in the trunk and upper and lower extremities. A pure generalized startle reflex is not always seen in both proximal and distal muscles.[41] Startle reflex activity is thought to result from the activation of reticulospinal motor tracts in the pontomedullary reticular formation (pmRF), which in the case of auditory startle, is triggered by direct synaptic activation from the cochlear nucleus.[194,386] Importantly, neurons of the pmRF are not modality specific, and therefore respond to different types of afferent information.[380] For example, startle reflexes can be elicited by acoustic stimuli,[42] tactile stimuli,[138] high intensity visual stimuli,[39] and by stimulation of the vestibular system via unexpected vertical drops of the body.[20,144] Startling stimuli do not only result in startle reflexes, but also in blink responses due to activation of the orbicularis oculi muscle. Pathways underlying blink responses are thought to be different from startle reflexes, involving neurons of the inferior colliculus and mesencephalic reticular formation.[169]

Habituation and prepulse inhibition are important characteristics of startle reflexes. Habituation of startle reflexes is observed when startling stimuli are given in repetition. Startle reflexes decrease in amplitude with repeated exposure, and eventually only the blink response will remain.[42] Habituation occurs most often after two to six presentations of a startling stimulus, and is presumably the result of synaptic depression at the pmRF.[69] Prepulse inhibition is the inhibitory effect of a weak sensory signal given 30-500 ms prior to the startling stimulus.[139,193,346] The neural pathways involved in prepulse inhibition likely include the pedunculopontine nucleus (PPN),[174,194] as studies in rats showed that PPN-lesions abolish prepulse inhibition.[345] For more

detailed information about prepulse inhibition and habituation of startle reflexes, we refer to excellent reviews by Valls-Solé and colleagues[359] and Carlsen and colleagues.[59]

Modulation of startle reflex expression by posture and gait

Studies that evaluated startle reflexes during gait and various postures have shown that the expression of startle reflex activity is modulated by afferent input. During gait, a phase dependent modulation of startle reflexes is observed, with more prominent startle reflexes in the stance leg compared to the swing leg.[260,314] Startle reflexes in lower leg muscles are more prevalent while standing compared to sitting, whereas rates of occurrence of reflex EMG activity in the sternocleidomastoid muscle do not differ between both postures.[41,89] Moreover, shorter latencies of reflex EMG activity in leg muscles are observed while standing (70-95 ms) compared to sitting (120 ms).[41,89] Finally, a recent study found that more subtle changes in posture, such as weight-bearing asymmetry, also influence startle reflex expression in leg muscles.[267] It has been suggested by these authors that afferent loading information plays a critical role in the observed modulation of startle reflexes. Such information is provided by Ib-afferents from Golgi tendon organs and cutaneous mechanoreceptors in the foot soles.[98,106,181] Modulation of startle reflex activity by afferent loading information likely takes place at the spinal cord, as studies in cats indicated that symmetrical volleys from the pmRF are gated at premotoneural level by spinal interneuronal networks.[102,316]

Modulation of posture and gait by startle reflexes

Startle reflexes likely contribute to the large amplitude postural responses that are observed when balance is perturbed unexpectedly or for the first time in a series of perturbations.[52,325] Amplitudes of postural responses to balance perturbations are significantly larger during the first trial compared to subsequent trials involving the same postural stimulus, a phenomenon known as first trial effect.[5] First trial effects are observed in whole body postural responses that occur when standing balance is perturbed,[5,24,70,185,279,280,347] but also in postural responses in neck and trunk muscles that occur during seated perturbations.[29,30] These first trial effects have been suggested to result from summation of startle reflex activity on the basic postural response.[29,52,249,325] This hypothesis is supported by three observations. First, postural responses habituate after the first trial in a series of repeated perturbations, just like habituation of startle reflexes.[52,280] Habituation of postural responses could as such consist of the extinction of startle reflexes, leaving only the postural response.[325] Second, early masseter activity after first trial perturbations might be indicative of a startle-like component contributing to balance responses,[370] as early activation of this muscle is indicative of the presence of a startle reflex.[42] A third observation in support of startle-like components contributing to balance responses relates to coherence in EMG activity between bilateral neck muscles during rear-end perturbations.[29]

An increased coherence in the 10-20 Hz bandwidth is observed after startling auditory stimuli, and is thought to represent increased reticulospinal activity.[143] During rear-end forward perturbations, an increased coherence in the 10-20 Hz bandwidth was seen during the first trial.[29] During subsequent habituated trials the synchrony between bilateral neck muscles decreased significantly, but reappeared when a startling acoustic stimulus was superimposed on the whiplash-like perturbation. Hence, these results also indicate a superimposition of startle reflex activity on the postural response during first trial perturbations.

The above observations raise the question whether the summation of startle reflex activity on the basic postural response results in functional benefits. The function of the startle reflex could lie in the rapid accomplishment of a defensive posture,[41,267] yet in modern life, summation of startle reflexes and postural responses might have disadvantages as well. The expression of startle reflexes following rear-end collisions has been suggested to contribute to increased forces and strains in neck tissues, leading to whiplash injuries.[217] In the same way, a startle reflex overlaid onto a normal postural response may be detrimental to balance, if it interferes with the planned amplitude, direction and inter-segmental coordination between limbs. Indeed, centre of mass deviations are larger during first trial perturbations compared to habituated postural responses.[280] Hence, the generalized startle reflexes induced by highly unexpected perturbations appear to reduce the net effect of appropriate corrective responses, which comes to the detriment of postural stability.

StartReact effect

Acceleration of reaction times by a startling stimulus

When a startling stimulus is presented in a reaction time task together with the imperative stimulus, reaction times are significantly accelerated, a phenomenon known as StartReact.[60,356,359] In addition to an acceleration of reaction times, an increase in EMG amplitudes and muscle force has also been reported for StartReact experiments.[10,198,293] The first StartReact experiments involved the acceleration of voluntary arm movements and voluntary rising onto the toes from a standing position.[355,356] These studies showed that a startling acoustic stimulus (SAS) accelerates movement latencies without changing the basic spatiotemporal pattern of muscle activation of the movement involved. Based on this observation, it was argued that SAS releases a pre-prepared motor program, rather than simply superimposing reflex activity on the intended movement. To test this hypothesis, Carlsen and colleagues evaluated StartReact effects on a series of arm movements to targets of 20, 40 and 60 degrees.[55] When a 20 degrees movement was prepared, a SAS resulted in a 20 degrees movement with its associated EMG pattern. However, when a 40 or 60 degrees movement was pre-planned, the EMG pattern was in accordance with the 40 or 60 degrees movement, providing evidence for release of

a pre-prepared motor program by a startling stimulus. This notion is further supported by the observation that a SAS, in the absence of the imperative stimulus, can elicit the planned movement, which is not observed when a SAS is given in isolation prior to the experiment.[198,357]

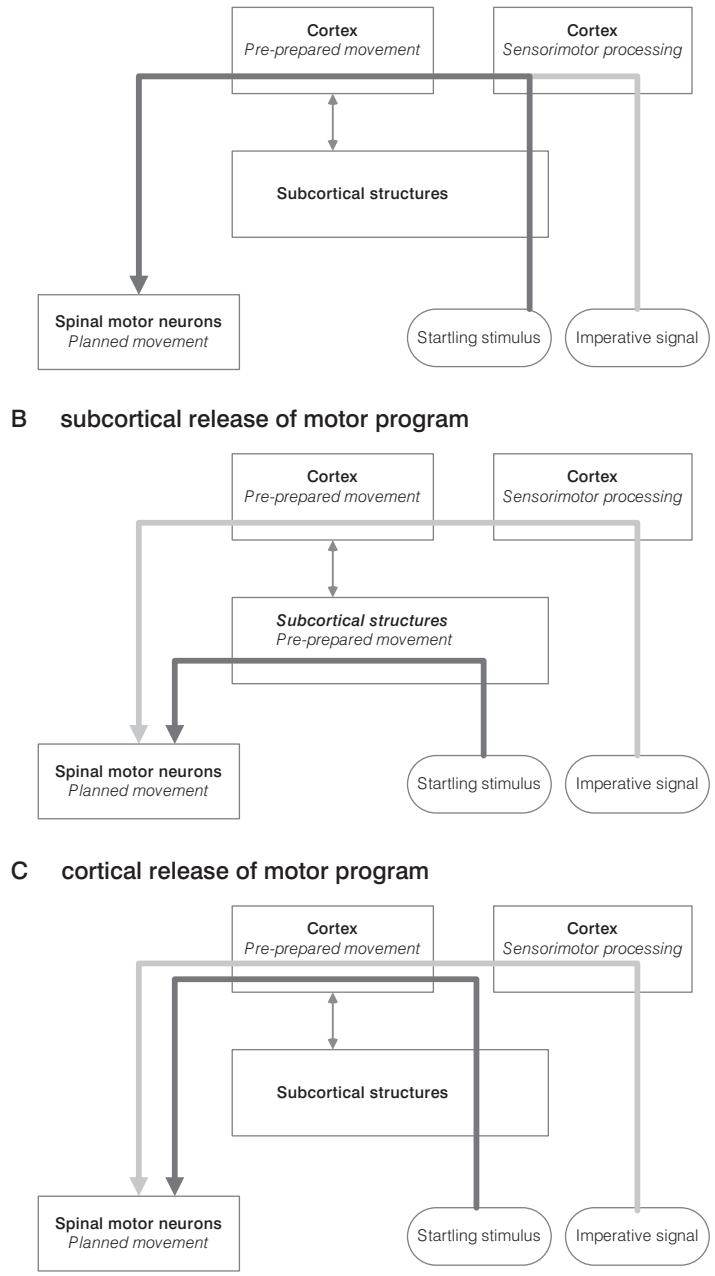
A second study by Carlsen and colleagues[54] evaluated the StartReact effect in a simple and choice reaction task. Since motor preparation does not generally occur during a choice reaction task, it was hypothesized that StartReact would not be observed during a choice reaction task. Indeed, a SAS only accelerated latencies during the simple reaction task and not during the choice reaction task. Subsequent studies did report acceleration of reaction times in some choice reaction tasks,[198,209,278] but this came at the expense of response errors. These observations suggest that subjects may prefer to preplan a movement sequence in advance, even if that plan may be potentially incorrect. Hence, the observed StartReact effects during choice reaction tasks also indicate that motor preparation is a prerequisite for the acceleration of reaction times by a SAS.

Mechanisms underlying StartReact

Although there is general consensus that StartReact is due to release of a pre-prepared motor program by a startling stimulus, the neural structures involved are still a matter of ongoing debate. Three hypotheses have been proposed to explain the mechanism underlying StartReact. First, a SAS could act as an additional stimulus on top of the imperative stimulus, thereby increasing the energy of the sensory input, a process known as intersensory facilitation.[255] Intersensory facilitation could subsequently result in an acceleration of sensorimotor coupling at the cortical level, resulting in accelerated release of motor programs, conveyed by the corticospinal tract (see Figure 1A). However, it has been reported that reaction time shortening during a StartReact experiment is not dependent on the intensity of the stimulus, but rather on whether the stimulus is perceived as startling.[56] Another argument against the intersensory facilitation hypothesis comes from the observation that a SAS can trigger the requested movement at similarly short latencies when applied in the absence of the imperative signal.[198,266,293,357]

The second hypothesis on StartReact proposes that a SAS directly releases a subcortically stored motor program,[55,356] which is conveyed by the reticulospinal tract (see Figure 1B).[310] This hypothesis has been proposed as latencies of the fastest StartReact effects seem to be too fast to involve the motor cortex.[356] Moreover, onsets of muscles involved in StartReact effects tend to have the same latency as those seen in a startle reflex, indicating conduction by the reticulospinal tract.[356] Recent evidence supporting this notion has been developed from studies using two clinical patient models. In chronic stroke patients, it was first found that StartReact responses are intact for elbow flexion and extension movements.[162] For elbow flexion, there were

Figure 1 Potential mechanisms underlying StartReact (in part modified from[3]).



no differences in either onset latency of muscle activation patterns between stroke patients and controls. During elbow extension, a SAS exhibited inappropriate activity in the flexors, but this diminished over time, leaving a normal muscle activation pattern. Likely, inappropriate startle reflex activity was seen during the first elbow extension trials, which habituated over time.[162] More recently, it has been shown that StartReact effects on hand extension movements are intact in most stroke patients as well.[165] These results can be interpreted in favor of a SAS releasing a motor program from a subcortical level, but one has to acknowledge that stroke patients have remaining cortical connections, which leaves the possibility that a SAS releases a cortically stored motor program conveyed by these residual corticospinal fibres (see below).

StartReact effects were also studied in patients with hereditary spastic paraplegia (HSP).[125,271] HSP is a disease characterized by retrograde axonal degeneration of the corticospinal tract,[38,177,253,281,289,312] while leaving the reticulospinal tract unaffected.[265,271] Typically, HSP in its pure form does not affect the corticospinal tract to the upper extremities[177,281,289,312] (see Figure 2A). StartReact effects in patients with pure HSP were compared to those in age-matched controls using a reaction task involving ankle dorsiflexion and a second reaction task involving wrist flexion.[271] Ankle

Figure 2A Schematic diagram of the neurologic disruption in patients with HSP. CST = corticospinal tract. RST = reticulospinal tract. In patients with HSP, the reticulospinal tract is intact, but there is retrograde axonal degeneration of the corticospinal tract (schematically represented by the dotted line). Typically, HSP in its pure form does not affect the corticospinal tract to the upper extremities.

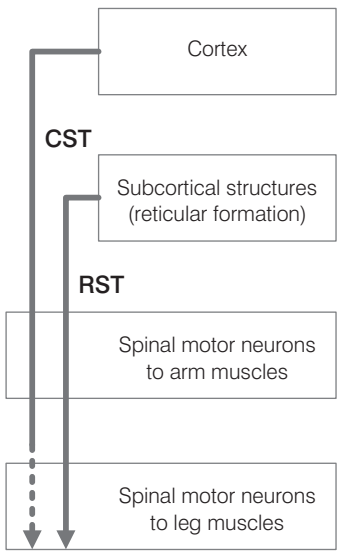
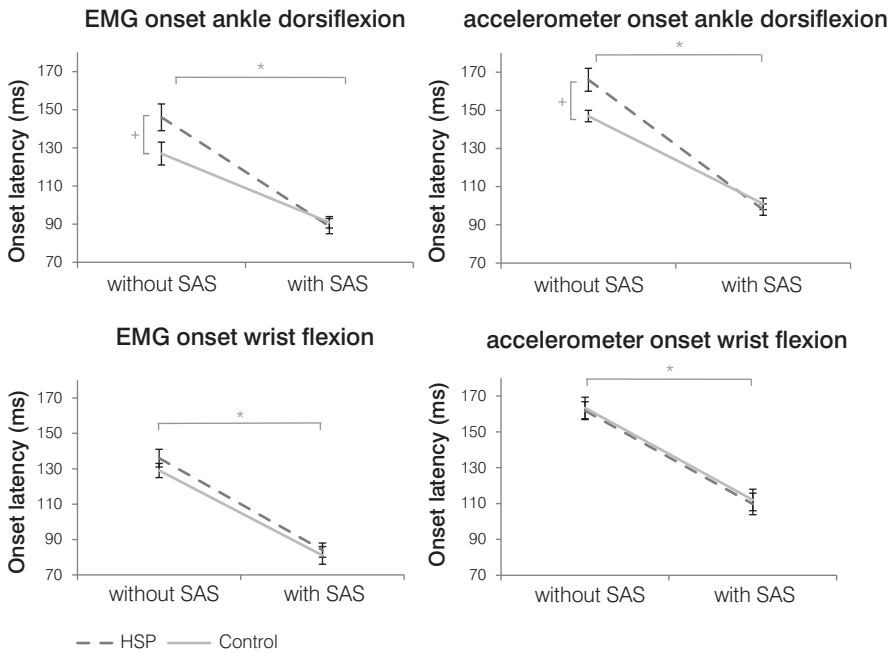


Figure 2B Mean onset latencies (SE) during the simple reaction time tasks involving voluntary ankle dorsiflexion (upper graphs) and wrist flexion (lower graphs) in patients with HSP and age-matched controls. * indicates significant differences between trials with and without a SAS. + indicates a s significant SAS x group interaction. This figure is reprinted from 271 in agreement with the journal's policy.



dorsiflexion reaction times were delayed in patients with HSP compared to controls, in line with delayed motor evoked potentials in tibialis anterior in response to supra-maximal transcranial magnetic stimulation (TMS). However, when the ankle dorsiflexion task was combined with a SAS, reaction times were accelerated in both patients with HSP and controls, but to a larger extent in patients with HSP, resulting in completely normalized EMG and movement onset latencies (see Figure 2B). When the reaction time task involved voluntary wrist flexion, no differences in onset latencies between patients with HSP and controls were observed, irrespective of the presence of a SAS. This observed pattern of results was interpreted by the authors in favor of a SAS releasing a subcortically stored motor program, conveyed by the reticulospinal tract. The third hypothesis on StartReact proposes that the SAS could act as a subcortically mediated trigger for a cortically stored motor program,[3,60] conveyed by the corticospinal tract (see Figure 1C). The latter hypothesis is supported by three recent

studies that evaluated the effect of TMS on the StartReact effect.[3,220,338] Alibiglou and Mackinnon showed that a single, suprathreshold pulse of TMS delivered over the primary motor cortex delayed reaction times to a startling stimulus.[3] These findings were replicated in a subsequent study that demonstrated longer delays in reaction times with shorter time between the TMS pulse and the startling stimulus.[338] Finally, Marinovic and colleagues[220] found that during preparation for anticipatory movements, the net excitability of the corticospinal pathway is enhanced shortly after a loud acoustic stimulus is presented.

Evidence for a cortical role in the StartReact effect is also provided by a recent study that evaluated movement-related EEG potentials during motor preparation. MacKinnon and colleagues showed that under preparatory conditions in which the timing of onset of the imperative cue could be predicted in advance, the presentation of a SAS could release a planned movement sequence as early as 1.5 seconds prior to the expected cue.[210] Importantly, the incidence of release significantly increased during cortical motor preparation (as indicated by the Contingent Negative Variation (CNV) in the movement-related EEG). For conditions in which the timing of the imperative cue could not be predicted, SAS-evoked reaction times of less than 100 ms were significantly less common and movement-related EEG potentials were markedly reduced compared to the condition in which the timing of the imperative cue could be predicted. These findings therefore suggest that motor preparation at the cortical level prior to an imperative stimulus sets a modulatory state for the early and unintentional release of the planned action.

Despite these seemingly contrasting hypotheses, however, involvement of the cortex in the StartReact effect and the suggested mechanism of a subcortical motor program being released by a SAS are not mutually exclusive. The delaying effect of TMS on SAS-induced acceleration of motor responses may be explained by the fact that TMS over the motor cortex not only has inhibiting effects on the cortical neurons, but also on cells within the reticular formation.[124] In addition, the observation that cortical motor preparation was associated with a greater probability of advanced release of the prepared movement[210] neither excludes a subcortical pathway underlying the StartReact effect. The modulatory state for the release of planned action may not be restricted to the motor cortex, as the incidence of SAS-induced startle reflexes also increases with greater motor preparedness.[58]

On the other hand, the completely normal SAS-induced reaction times in people with cortical lesions or corticospinal degeneration do not seem to be compatible with a transcortical pathway.[162,165,271] Hence, the current body of evidence seems to favor the hypothesis of the SAS releasing a prepared movement through a subcortical pathway. However, we suggest the cortex to play a critical role in the StartReact phenomenon by altering the state of the involved subcortical structures, which is in line with the

model recently proposed by Shemmell.[323] Future studies need to further unravel the exact neural pathways involved in StartReact. The pmRF may be regarded as a candidate subcortical structure in this respect, as it not only constitutes a key structure in the startle reflex circuitry, but has also been demonstrated in studies in monkeys and cats to be involved in motor preparation.[45,315]

Differences between startle reflexes and StartReact

There are several observations that suggest that startle reflexes and StartReact effects are at least partly dissociated. First, while prepulse inhibition modifies startle reflexes, it does not appear to influence the StartReact effect.[358] Second, startle reflexes in the sternocleidomastoid muscle do not seem to be a requirement for StartReact effects. Although Carlsen and colleagues[56] reported an attenuation of StartReact effects when no reflex activity was observed in the sternocleidomastoid muscle, several other studies have not found a significant relationship between reflex activity in the sternocleidomastoid muscle and StartReact, both for experiments involving simple reaction time tasks,[271] and for tasks involving gait and postural resp onses.[52,209,266,271,300,307] A final observation in support of dissociated processes between startle reflexes and StartReact comes from a study in patients with Parkinson's disease and severe freezing of gait.[349] In these patients, StartReact effects and startle reflexes were reported to be absent, but deep brain stimulation of the PPN selectively restored StartReact effects, while leaving the impairment in startle reflexes unchanged.

Interestingly, the StartReact effect has also been observed in response to lower-intensity stimuli not usually regarded as startling in nature, with the likelihood of early release of the prepared movement being related to the strength of the stimulus.[88] StartReact paradigms typically apply acoustic stimuli at ~120 dB, which intensity is large enough not only to release the prepared movement, but also to overcome the neural thresholds in the startle circuitry at the pmRF, resulting in a startle reflex. In the study of Delval and co-workers,[88] however, an 80 dB acoustic stimulus resulted in a fair proportion (29%) of trials with early release of the prepared motor response, and it also evoked a startle reflex in SCM in 18% of the trials (with 27% overlap between these events). These observations indicate that the preparatory motor state dramatically reduced the neural thresholds of StartReact and startle reflex circuitry (described as a progressive 'releasing of the brakes').[210] The relatively small proportion of trials with both early release of the prepared motor response and a startle reflex in SCM, however, suggest that the neural thresholds of these circuits may differ to some extent. This notion further supports the postulated dissociation between startle reflexes and the StartReact phenomenon.

StartReacts effects observed during gait initiation and online step adjustments

Following the observation of startle-induced acceleration of simple reactive movements, several groups investigated whether StartReact effects can also be induced during more complex movements, such as gait under a variety of conditions involving preplanning of movement. It was shown that StartReact was also applicable to two aspects of gait: gait initiation and online step adjustments.[209,293,300] Gait initiation involves anticipatory postural adjustments (APAs) that propel the body mass forward and laterally to unload the swing leg.[223] Interestingly, these APAs can be significantly accelerated by a startling stimulus,[88,209] which suggests postural preparation before a step is made.

The application of StartReact has more recently been used to study gait initiation in patients with Parkinson's disease (PD). Impairments during gait initiation are common in patients with PD and include reduced step length and small APAs.[46,131,232] In the more advanced stages, freezing of gait can emerge. Freezing of gait is characterized by sudden, relatively brief episodes of an inability to step or by extremely short steps.[275] StartReact experiments have been used to study whether deficient motor preparation contributes to freezing of gait. Intact startle-induced acceleration of APAs has been observed in PD-patients without freezing of gait,[119,269,307] indicating preserved motor preparation. Interestingly, reduced StartReact effects of APAs have been reported in PD-patients with freezing of gait.[269] Hence, in these patients, the APA to initiate a step may not be properly prepared in advance. An alternative explanation for the reduced StartReact effect in freezers may be that the reflexive release of motor responses is deficient due to subcortical structures being less responsive to excitatory stimuli. Although not confirmed yet, the finding of reduced StartReact effects in freezers could be relevant to the mechanisms underlying freezing of gait. Subcortical structures, in particular the pmRF and PPN, are involved in the integration of APAs with subsequent stepping movements.[199,246,275,317] Deficiencies in APA representation or release at the subcortical level could hamper the integration with subsequent steps, finally leading to the freezing of gait. However, future studies are needed to investigate this hypothesis.

As mentioned above, StartReact effects are also observed during online step adjustments.[293,300] Reynolds and Day evaluated the effect of a SAS on stepping adjustments in the medial or lateral direction,[300] responses that are thought to be organized at subcortical level.[301] Interestingly, startle-induced shortening of reaction times was observed, even though the direction of the adjustments was not known in advance. Queralt and colleagues[293] studied step adjustments in the sagittal plane in response to a sudden obstacle while participants walked on a treadmill. In their study, obstacles were released in specific moments of the step cycle, accompanied by a SAS (40 ms after obstacle release) in 25% of the trials. Contact of the foot with

the obstacle could be avoided by either lengthening or shortening of the ongoing stride, the likelihood of which depended on the moment of obstacle release. Results showed that both shortening and lengthening of the stride was accelerated in trials in which a SAS was applied. Step adjustments in the same direction were also observed when the SAS was presented in the absence of obstacle release, but at the same moment in the step cycle. The latter observations suggest that during gait, motor preparation for online gait adjustments takes place in advance.

We hypothesize that a startling stimulus releases a motor program for the online step adjustment, which is further modulated at the level of the spinal cord, depending on the phase of the gait-cycle, yielding either shortening or lengthening of the stride. This hypothesis is supported by studies in cats, that reported that descending activity can be manipulated by spinal structures, providing activation or suppression of motoneurons, depending on the phase requirements.[102] The observation that directionally appropriate on-line step adjustments to sudden medial or lateral target jumps were also susceptible to StartReact,[300] raises the intriguing question whether there may be a subcortical pathway for visual control of the lower extremity. The role of the collicular circuitry in the generation of arm reaching movements is well established and a link to leg movements has been suggested (through projections to the cuneiform nucleus[284]).

StartReact effects and postural responses

The relationship between StartReact and postural responses has been explored in two ways. First, it has been investigated whether postural perturbations can induce StartReact effects. As outlined in paragraph 2.3 of this review, there is evidence for superposition of startle reflex activity on the postural response, in particular following the very first trial of an unexpected balance perturbation. The suggestion that a postural perturbation can act as a startling stimulus was recently tested in two studies that applied a postural perturbation to induce StartReact effects. Indeed, whole body balance perturbations were shown to elicit StartReact effects on wrist extension movements.[52] Similarly, rapid perturbations of arm posture induced StartReact effects on elbow extension movements as well.[297] Hence, these observations provide further evidence for the startling nature of unexpected postural perturbations.

Second, studies have investigated whether motor preparation also takes place for postural responses to external balance perturbations, yielding these responses susceptible to acceleration by a SAS. In contrast to voluntary reactions in response to an imperative auditory or visual stimulus, automatic postural responses do not involve transcortical pathways,[176,292,348] but are likely encoded by assemblies of neurons in the pmRF.[337] However, three parallel studies[50,51,266] demonstrated that these automatic postural responses can still be accelerated by a SAS. Campbell and colleagues evaluated the effect of auditory stimuli after repeated cued sideways

perturbations. A nonstartling auditory tone was able to evoke a postural response in the absence of a perturbation,[51] indicating the presence of a classically conditioned response. Interestingly, a SAS induced significantly earlier onsets of conditioned postural responses compared to the auditory tone, hinting towards advanced preparation of postural responses. A second argument for such preparation came from a study that evaluated whether automatic postural responses to balance perturbations can be accelerated by a SAS.[266] In this study, postural responses to backward and forward perturbations were induced by a balance platform that could translate in the forward or backward direction respectively.[264] In 25% of balance perturbations, a SAS was given at the start of platform translation. Postural responses to backward perturbations could be significantly accelerated by a SAS, both when a backward perturbation was expected, but also when perturbation direction was not known in advance. In line with the study of Campbell et al.,[51] a SAS yielded postural responses at similar latencies in the absence compared to those in the presence of a balance perturbation, and both these latencies similarly scaled with perturbation intensity. Interestingly, a SAS did not shorten postural responses to forward perturbations,[266] this finding being replicated in a subsequent study by the same group.[268] While the latter study demonstrated a directional specificity of StartReact effects on postural responses in the sagittal plane, subsequent work has shown that postural responses can be accelerated by a startling stimulus in the lateral direction as well.[50] Hence, backward and sideways perturbations are susceptible to StartReact, while forward responses are not. The mechanism responsible for this directional specificity of StartReact in postural responses is not yet understood. It may be that postural responses in forward, backward and sideways directions involve different neural circuits, with startle circuits selectively interacting with postural responses to recover from backward and sideways perturbations.[266]

Conclusion

Recent work on startle reflexes and StartReact has provided important new insights into the neural mechanisms underlying startle reflexes and StartReact, and have contributed to our understanding of control of posture and gait. First, studies using patients with corticospinal lesions support a mechanism for StartReact that incorporates a motor program that is stored in subcortical centres and triggered by a startling stimulus. Experiments using TMS suggest that the cortex plays a critical role in the StartReact phenomenon, possibly by altering the state of the involved subcortical structures. Second, it is recognized that the behaviors susceptible to StartReact have expanded considerably, now also involving postural control and gait. Originally this work was concentrated on simple voluntary movements, but in recent

years it has been shown that gait initiation, online step adjustments and postural responses to backward and sideways perturbations can be speeded up by a startling stimulus as well. This indicates that advanced motor preparation takes place for these elements of postural control and gait. This preparation presumably involves a close interaction between cortical and subcortical structures. However, the exact neural pathways involved in advanced motor preparation of posture and gait remain to be unraveled by future studies. In light of our understanding of the mechanisms involved in StartReact, these results support the growing body of evidence for the cortical and subcortical contributions to balance and gait.[1,49,176,221,273,292,299] Third, several studies indicate a superimposition of startle reflex activity on the postural response during first trial balance perturbations. The summation of startle reflex activity on the postural response can be detrimental for postural stability if it interferes with the amplitude and direction of the balance correcting response. The growing number of studies in this area of research has also raised several new questions on posture and gait control, including the mechanisms underlying the directional specificity of SAS-induced acceleration of postural responses and the susceptibility of visually-guided step adjustments to StartReact, which are suggested topics for further research.

12

Summary and general discussion

Summary

The aim of this thesis was to study the mechanisms underlying deficits in motor control in both pyramidal and extrapyramidal neurodegenerative diseases, with an emphasis on gait and balance impairments. In particular, I was interested in the role of brainstem structures in impaired motor control. A key method that was used to study motor control was the StartReact paradigm.[359] First, unaffected motor control in healthy humans was investigated. Then, gait and balance impairments in patients with hereditary spastic paraplegia (HSP, a pyramidal neurodegenerative disease) and Parkinson's disease (PD, an extrapyramidal neurodegenerative disease) were explored. Here, the findings of this thesis are summarized. In the section 'General discussion', the results are placed in a broader perspective and directions for future research are given.

Part 1: Healthy subjects

Influence of loading on startle reflex expression in the lower extremity

In **chapter 2**, I studied why the occurrence of startle reflex activity in distal leg muscles varies according to posture. The startle reflex is an involuntary reaction to sudden unexpected sensory input and is the fastest generalized motor reaction of humans and animals. The startle reflex is the result of a downward volley from the pontomedullary reticular formation (pmRF, located in the brainstem), conveyed by the reticulospinal tract.[194,386] Startle reflex activity is most prominent in the sternocleidomastoid muscle, from where it radiates to distal muscles.[41] The occurrence of reflex activity in distal leg muscles varies according to posture, and is larger in a standing position compared to sitting relaxed.[41,89] I hypothesized that sensory input from load receptors modulates the occurrence of startle responses in leg muscles. To test this hypothesis, sudden startling auditory stimuli were administered to eleven healthy subjects while (1) sitting relaxed, (2) standing relaxed, (3) standing while bearing 60% of their weight on the right leg, (4) standing while bearing 60% of their weight on the left leg, and (5) standing with 30% body weight support ('bilateral unloaded'). Electromyography (EMG) data were collected from both tibialis anterior (TA) and the left sternocleidomastoid muscles. In the TA, startle responses occurred much more frequently during normal standing compared to both sitting and bilateral unloading. In the asymmetrical stance conditions, startle responses in the TA were more common in the loaded leg compared to the unloaded leg. Hence, the occurrence of startle responses in the leg muscles was strongly influenced by load. In line with the hypothesis, it is likely that information from load receptors influences startle response activity. It was suggested that, in a stationary position, startling stimuli result in a

descending volley from brainstem circuits, which is gated at spinal level by afferent input from load receptors. Furthermore, it was suggested that one of the functions of the startle reflex lies in rapidly accomplishing a defensive stance with maximum postural stability. To accomplish maximal postural stability, leg muscle activation is only useful when someone is actually standing, and in case of an asymmetrical weight distribution of the legs, activation of the muscles in the loaded leg is most important.

The expression of startle reflex activity in distal leg musculature depends on the amount of loading on a leg. One of the functions of the startle reflex lies in rapidly accomplishing a defensive stance with maximum postural stability.

StartReact and postural responses

Startling auditory stimuli (SAS) can accelerate reaction times when delivered simultaneously with an imperative cue in a reaction time task, a phenomenon known as 'StartReact'.[359] In **chapter** 3, I investigated whether postural responses to balance perturbations can also be accelerated by a SAS. Balance was perturbed in eleven healthy participants, and these perturbations were combined with a SAS in 25% of trials. The direction and magnitude of the perturbations was varied, as well as the prior knowledge of perturbation direction. Perturbation trials were interspersed with SAS-only trials.

Postural responses to backward perturbations were significantly fastened as well as strengthened by a SAS, irrespective of prior knowledge of the perturbation direction. A SAS did not shorten postural responses during forward perturbations. In fact, a SAS resulted in more prevalent responses in tibialis anterior (prime mover of backward postural responses) at reduced onset latencies. In addition, during 'SAS-only' trials, muscle responses were more often seen in tibialis anterior and rectus femoris compared to gastrocnemius. Finally, an increase in perturbation magnitude resulted in faster responses, with and without a SAS, particularly for backward perturbations. These very consistent directional effects of the SAS as well as of the perturbation magnitude suggested that postural responses to forward and backward perturbations involve different neural circuits. I suggested that a SAS might be able to trigger a default response protecting against backward loss, thereby bypassing afferent input. Finally, it was hypothesized that there is a possible involvement of startle circuits in responses to recover from backward perturbations.

Postural responses to forward and backward perturbations probably involve different neural circuits. A startle is able to trigger a postural response protecting against backward balance loss.

Facilitation of subcortical motor responses by tDCS

In **chapter 4,** we investigated whether it is possible to facilitate subcortical structures, in particular the reticular formation using transcranial direction current stimulation (tDCS). The effect of tDCS was assessed on two responses that are evoked from subcortical structures; (1) the StartReact effect, and (2) automatic postural responses to external balance perturbations. Ten healthy adults were measured on two different measurement sessions in which they first received anodal-tDCS (15 minutes, 2 mA) or sham-tDCS in a counterbalanced order. The anodal electrode was placed over the non-dominant motor region, the reference electrode over the contralateral supraorbital region. After stimulation, participants were instructed to respond as rapidly as possible to a visual imperative stimulus in three separate conditions: dorsiflexion of the dominant or non-dominant ankle, or flexion of the dominant wrist. Furthermore, postural responses to forward and backward balance perturbations were evaluated. A SAS (116 dB) was delivered simultaneously with the imperative stimulus and balance perturbations in 25% of trials. Electromyographic responses and accelerometer data were collected. A SAS accelerated latencies of the simple ballistic movements, and the responses to backward balance perturbations. In all tasks, responses were significantly faster after anodal-tDCS compared to sham-tDCS, both in trials with and without a SAS. These findings strongly suggested that subcortical structures in humans, in particular the reticular formation, can be facilitated by tDCS.

Subcortical structures in humans can be facilitated by transcranial direct current stimulation.

Part 2: Hereditary spastic parapegia

Underlying mechanisms of the StartReact effect

In **chapter 5**, I explored the underlying mechanisms of the StartReact phenomenon. Previously, three mechanisms had been proposed to explain the occurrence of the StartReact effect. First, a SAS could act as an additional stimulus on top of the imperative stimulus, thereby increasing the energy of the sensory input, resulting in an acceleration of sensorimotor coupling. This hypothesis, known as intersensory facilitation,[255] involves the corticospinal tract, both in trials with and without a SAS. A second hypothesis was that a SAS acts as a subcortically mediated trigger for a cortically stored motor program, which would involve the corticospinal tract and reticular-cortical pathways.[3,60] Third, a SAS could yield accelerated motor responses through a release of a subcortically stored pre-prepared motor program,[55,356] which is conveyed by the reticulospinal tract.[310,359] To distinguish between these hypotheses, the StartReact phenomenon was examined in patients with HSP, a disease characterized by retrograde axonal degeneration of the corticospinal tract and the

posterior spinal columns.[228] Twelve patients with autosomal dominant pure HSP and 12 matched healthy controls were instructed to respond as rapidly as possible to a visual imperative stimulus in two different conditions: dorsiflexion of the dominant ankle or flexion of the dominant wrist. In 25% of trials, a SAS was delivered simultaneously with the imperative stimulus. Prior to these tests, subjects received five SAS while standing to verify normal function of the reticulospinal tract in HSP. I first showed that the reticulospinal tract does not seem to be affected in HSP patients, as occurrence and latencies of startle reflexes did not differ from controls. HSP patients showed delayed reaction times during ankle dorsiflexion and normal latencies during wrist flexion, reflecting the retrograde degeneration of the cortico-spinal tract. Administration of SAS accelerated ankle dorsiflexion and wrist flexion in both groups. However, during ankle dorsiflexion, a larger acceleration was seen in the HSP patients, which completely normalized their latencies. These findings suggested that a SAS accelerates reaction times through a release of a subcortically stored motor program, conducted by the reticulospinal tract. In chapter 5, it was discussed which exact subcortical structures might be involved in the StartReact effect. Studies in monkeys and cats have identified the pontomedullary reticular formation (pmRF) as one of the subcortical structures that subserves motor preparation. As the pmRF is also a key structure in the startle reflex circuitry, it may play a pivotal role in the release of pre-prepared motor programs, resulting in the StartReact effect.

A startle is able to accelerate reaction times (StartReact effect) by directly releasing a subcortically stored motor program, conveyed by the reticulospinal tract

Balance impairments in HSP

In **chapter 6**, I investigated the mechanisms underlying balance impairments in HSP. I hypothesized that delayed postural responses contribute to the balance problems in HSP. To test this hypothesis, eighteen patients with HSP and nine healthy controls stood on a balance platform and were instructed to counteract forward and backward balance perturbations, without taking a step or grabbing a handrail. Patients with HSP were less successful than controls in maintaining balance following backward and forward perturbations. Furthermore, latencies of postural responses were significantly delayed in patients with HSP, by 34 ms in gastrocnemius following forward, and by 38 ms in tibialis anterior following backward perturbations. We reasoned that the delayed postural responses could be the result of a delay of signals in the afferent or efferent tract, or both. Afferent input following an externally imposed balance perturbation is conducted by the posterior spinal columns and integrated at the level of the brainstem and cortex. The first efferent signals contributing to the postural response arise from the brainstem and are conveyed by the reticulospinal

tract. To distinguish between a possible delay of signals in the afferent (posterior spinal columns) or efferent (reticulospinal) tracts, balance perturbations both with and without a concurrent SAS were used. Twelve patients with HSP and nine controls received backward perturbations, while a SAS accompanied onset of platform motion in 25% of trials. Combining balance perturbations with a SAS restored normal latencies, suggesting that conduction of efferent signals (the reticulospinal tract) is normal. I therefore suggested that the delayed postural responses in HSP are caused by slowed conductions times in the posterior spinal columns.

Delayed postural responses contribute to balance impairments in patients with hereditary spastic paraplegia. Slowed conduction of afferent signals in the posterior spinal columns causes the delay in postural responses.

Part 3: Parkinson's disease

Dynamic posturography to study balance impairments in PD

Chapter 7 is a narrative review on recent developments in the field of dynamic posturography. Novel moveable platforms can deliver 'real-life' balance perturbations, permitting study of falls under circumstances that resemble everyday life. It was highlighted how these recent innovations can help to understand the pathophysiology of postural instability in PD. Dynamic posturography studies have already shown that patients with PD have fundamental problems in the scaling of their responses. It was stated that future studies could further explore the balance correcting steps in PD and the presumed interaction between startle pathways and postural responses.

Recent innovations in posturography allow for delivering 'real-life' balance perturbations, permitting study of falls under circumstances that resemble everyday life.

Underlying mechanisms of FOG

In **chapter 8**, I studied freezing of gait (FOG) in patients with PD. FOG is an episodic gait disorder where patients experience sudden and often unexpected episodes during which their feet feel like 'being glued to the floor' while their trunk tends to move forward.[275] The underlying mechanisms of FOG are poorly understood, but emerging evidence suggests that dysfunction of the pedunculopontine nucleus (PPN) and pontomedullary reticular formation (pmRF) play a role in causing FOG.[275] The function of these upper brainstem structures was examined by the StartReact effect. In freezers, the StartReact effect is disturbed for elbow flexion,[349] but it remains unclear whether and how a disturbed StartReact effect in a simple ballistic movement

of the upper extremity relates to FOG. I reasoned that demonstration of an impaired StartReact effect in a gait-related task would provide stronger support for upper brainstem dysfunction in FOG. Twenty-six patients with PD (12 freezers and 14 non-freezers) and 15 healthy controls performed two tasks: (1) rapid gait initiation in response to an imperative 'go' signal; and (2) a control condition, with a simple reaction time task involving dorsiflexion of the foot. This second condition served as a positive control, to confirm prior findings of a disturbed StartReact effect in a simple ballistic movement. During both tasks a SAS was combined with an imperative 'go' signal in 25% of trials. Primary outcomes were the onset latency of the tibialis anterior muscle, step length and amplitudes of anticipatory postural adjustments (APAs). In controls, the SAS accelerated gait initiation and reduced the onset of tibialis anterior activity during ankle dorsiflexion. This acceleration was intact in non-freezers, but was significantly smaller in the freezers. Independent of the occurrence of a startle, freezers showed a reduced length of the first step compared to non-freezers and controls. In conclusion, the StartReact effect was diminished in freezers during gait initiation and voluntary ankle dorsiflexion, which probably reflects a deficient representation of motor programs at the brainstem reticular level due to dysfunction of the PPN, the pmRF, or both. These brainstem structures are presumably involved in integrating APAs with subsequent stepping movements. I suggested that with time-varying demands, these structures may no longer be able to coordinate the integration of anticipatory postural adjustments with steps, leading to FOG-episodes.

A reduced ability of upper brainstem structures to integrate APAs with subsequent stepping movements may underlie freezing of gait in patients with Parkinson's disease.

StartReact differentiates between gait freezing and postural instability

The frequent co-existence of postural instability with FOG raises the possibility of shared pathophysiology. In chapter 8, I showed that dysfunction of upper brainstem structures might contribute to the causation of FOG. In **chapter 9**, I evaluated whether dysfunction of these structures contributes to postural instability as well. To this aim, I contrasted patients with and without postural instability and with and without FOG with respect to the StartReact effect on postural responses to backward balance perturbations. Twenty-five patients with idiopathic PD (11 with postural instability (6 freezers); 15 without postural instability (5 freezers)) and 15 healthy control subjects participated. Postural responses were tested by translating a balance platform in the forward direction, resulting in backward balance perturbations. In 25% of trials a SAS was accompanied with the start of the balance perturbation. The StartReact effect was also evaluated in a simple reaction time task involving ankle dorsiflexion. The accelerating effect of the SAS was intact in patients with postural

instability, but was attenuated in the freezers. This was seen for both postural responses and the ankle dorsiflexion movement. The amplitude of the automatic postural responses and the length of the first balance correcting step were smaller in patients with postural instability compared to patients without postural instability, but did not differ between freezers and non-freezers. Due to these consistent differences, I suggested that mechanisms underlying FOG and postural instability are at least partly different. I concluded that underscaling of both the automatic postural response and the first step to recover from balance perturbations contributes to postural instability in PD. The attenuated StartReact effect was only seen in freezers and likely reflects inadequate representation of motor programs at upper brainstem level.

Mechanisms underlying postural instability and freezing of gait are at least partly different. Underscaling of both automatic postural responses and balance correcting steps contributes to postural instability in PD.

A treatment algorithm for freezing of gait

Treatment of FOG is important because it is one of the most important risk factors for falls in parkinsonism, and a source of great disability to patients. However, many clinicians find it difficult to treat FOG in clinical practice, and this challenge is compounded by the lack of clear treatment protocols. To address this widely felt need, a practical algorithm for the medical and non-medial management of FOG was presented in **chapter 10**. All recommended interventions are based on evidence whenever this was available. For this purpose, I first classified the level of evidence underlying the available interventions for FOG. Otherwise the recommendations reflect practice-based evidence that is supported by the experience of the authors. Further work must formally establish the actual merits of this new treatment protocol. Pending such evidence, this protocol can support clinicians in their current management of FOG.

We present a practical algorithm for the medical and non-medical management of FOG. This protocol can support clinicians in their management of FOG.

Part 4: Overview

What startles tell us about control of posture and gait

Recently, there has been an increase of studies evaluating startle reflexes and StartReact, many of those delivered while subjects were performing tasks involving

postural control and gait. These studies have helped to improve our understanding on the neural mechanisms underlying startle reflexes and StartReact, and also provided insight on the neural control of posture and gait. In **chapter 11**, I provided a comprehensive review on startle reflexes and StartReact and their interaction with posture and gait.

General discussion

Here, the neural circuits and pathways involved in gait and balance control will be discussed first. I will explore how dysfunction in these neural circuits can result in gait and balance disorders in PD. Furthermore, I will elaborate how future studies can contribute to improved medical and non-medical management of FOG. Then, the focus will be on the role of the reticular formation in motor control and its potential compensatory role in motor recovery after pyramidal lesions. Finally, I will elaborate on the mechanisms and potential applications of the StartReact paradigm.

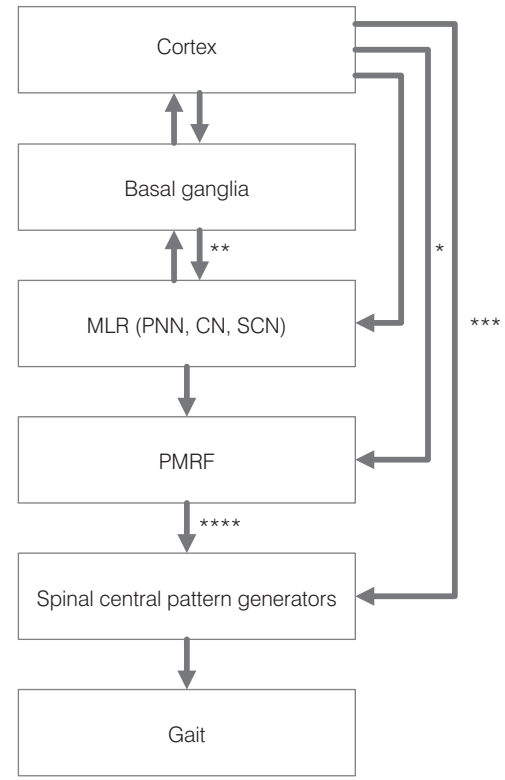
Gait and balance control

Neural circuits involved in gait

Gait requires stepping movements, which are generated in the spinal cord by central pattern generators (see Figure 1).[145] These central pattern generators receive descending signals from supraspinal structures, which are essential to initiate and modulate the stereotyped walking pattern.[275] Anticipatory postural adjustments (APAs) are required to maintain balance when stepping movements are being made.[223] APAs shift the body weight laterally and forward to unload the stepping leg, where after a step can be performed.[47] The integration of APAs and steps presumably involves the pontomedullary reticular formation (pmRF) and the mesencephalic locomotor region (MLR), both located in the brainstem.[275,317] The MLR consists of different nuclei, including the pedunculopontine nucleus (PPN), cuneiform nucleus (CN), and subcuneiform nucleus (SCN).[275] The distinct role of these nuclei in the integration of APAs and steps is yet unknown. The basal ganglia and the cortex, in particular the supplementary motor area (SMA), are involved in the scaling of APAs and stepping movements.[148,329] As the cortex and basal ganglia are involved in the scaling of the gait pattern, they are essential in the fine regulation of gait. In addition to the SMA, several other cortical regions are required for normal gait.[107] The frontal cortex is one of these, as gait requires cognitive processes such as attention, motivation and planning.[43]

As shown in Figure 1, cortical structures give input to all the subcortical and spinal structures involved in gait. Importantly, the MLR and pmRF also receive descending input from the basal ganglia via striatoreticular pathways.[104] Moreover, the spinal

Figure 1 Neural circuits involved in gait. MLR = mesencephalic locomotor region, PPN = pedunculopontine nucleus, CN =cuneiform nucleus, SCN = subcuneiform nucleus, pmRF = pontomedullary reticular formation, CPG = central pattern generator. * = cortico-reticular tract,**=striatoreticular tract, *** = corticospinal tract, **** reticulospinal tract. This figure is modified from Nutt et al., Lancet Neurol, 2011[275].



pattern generators and spinal motorneurons also receive input from the pmRF via the reticulospinal tract. Hence, gait is not only the result from corticospinal projections to spinal pattern generators, but heavily relies on subcortical input to the spinal cord.

It must be noted that normal gait is not possible without sound afferent feedback. All the structures described above receive sensory input. Using sensory feedback, the neural structures are able to modulate their output and thereby the gait pattern.[275,327]

Freezing of gait

FOG is most likely not the result of dysfunction at one neural structure involved in gait, but involves dysfunction and insufficient compensation at multiple levels.[275] In line with this idea, a recent review termed FOG the 'ultimate break in the network that

controls gait'.[43] In chapter 8, I have suggested that an inability of the pmRF and PPN to integrate APAs with subsequent stepping movements could contribute to the causation of FOG. This integration seems to be critical in freezers because of their reduced step length and small APAs. Both the reduced step length and smaller APAs have been attributed to reduced brain activity in the SMA.[148,329] I have suggested that when the pmRF and/or PPN are no longer be able to coordinate the integration of APAs with steps, FOG can emerge. I hypothesize that freezers are partly able to compensate for these deficits by heightening their attention. This could explain why FOG is hard to evoke in clinical practice or in a research laboratory,[234] situations that result in a higher attention and arousal. In line with this idea, it has been found that reduced executive functioning is associated with FOG.[7] Presumably, in patients with reduced executive functioning, less compensation is possible compared to patients without cognitive deficits, especially during challenging walking conditions such as turning or walking with short steps.

An important question for future studies is whether there is one pathophysiological mechanism underlying FOG, or whether it involves a combination of distinct patho-physiological phenomena. Freezing of gait has varied clinical patterns,[313] and there are several hypotheses on the mechanism underlying FOG.[275] I hypothesize that a reduced ability of brainstem structures to integrate APAs with subsequent stepping movements could contribute to FOG in a part of freezers, but not in all. This idea is in line with recent observations of our group on the effect of a walk-bicycle on the occurrence of FOG.[341] The walk-bicycle is a bicycle without pedals and a low seat that allows stepping movements, without the need to make an APA. As there is no need to couple APAs with subsequent steps, we hypothesized that the walk-bicycle would reduce the occurrence of FOG-episodes. We found that it reduced the occurrence of FOG-episodes in a relatively large percentage of freezers, but also increased FOG in some patients.[341] This observation corresponds with studies reporting on the effect of cueing on the occurrence of FOG.[257] The study on the walk-bicycle and those on cueing effects may suggest that the pathophysiological or compensatory mechanisms underlying FOG are not always the same in all patients. Future studies need to further investigate this hypothesis. To this aim, it will be important to carefully observe the clinical pattern seen in FOG. Moreover, it would be beneficial to include a relative large group of freezers, so that potential subgroups can be identified.

With respect to the clinical management of FOG, I hope that the practical algorithm described in chapter 10 will offer some support to clinicians in daily clinical practice. However, the level of evidence underlying several steps in our treatment algorithm is currently limited, and further investigation is needed. Randomized clinical trials are

needed that include FOG not just as one of many outcomes, but rather as the primary outcome. These future studies should include patients with dopamine-responsive, dopamine-induced and dopamine-resistant FOG, on the basis of unequivocal therapeutic responses obtained during history taking and – if needed – on the basis of observation of FOG before and after a challenge with a supramaximal levodopa dose before inclusion.[112,141] I recommend inclusion of patients whose FOG has been confirmed during neurological examination by an experienced observer (the so-called 'definite freezers').[214] Future studies should use a combination of both subjective assessment (using the validated FOG questionnaire)[258] and neurological examination (which should always include an assessment of rapid turning in place).[332,334] However, even this combination of tests might miss relevant FOG episodes in the patient's own home environment, highlighting the need for development of new measures that quantify the overall amount of FOG across the day. An interesting development is the introduction of wearable sensors (accelerometers or goniometers)[235,374] and perhaps even ambulatory electromyography[73] that might enable objective, continuous and quantitative detection of FOG during daily life. Although the initial findings with use of such sensors is promising,[212] their sensitivity and specificity are imperfect. Further work is therefore needed to identify which type of sensor, which number of sensors, and which positions give the best diagnostic yield for use in future clinical trials.
Development and assessment of new, more effective therapeutic approaches is needed, including pharmacological approaches (in particular non-dopaminergic drugs) and non-pharmacological approaches (such as visual cues provided by smart-glasses). Further investigation of the effect of amantadine on dopamine-responsive FOG and study of the effect of methylphenidate on dopamine-resistant freezing might be worthwhile. Surgical interventions for PD patients are developing at a rapid pace, with beneficial, and sometimes adverse effects on gait;[118,121] the challenge is to identify which targets and which stimulation protocols offer the greatest improvements in FOG for the different subtypes of FOG. In the speciality of physiotherapy, an interesting challenge is to ascertain whether cueing can be delivered safely and effectively in an on-demand manner –ie, with external cues being delivered only at a time when they are needed most. This challenge depends on development of reliable measures of FOG during free walking and, especially, of early markers that signal the nearby development of a new FOG episode. Initial research in this speciality is promising,[212] but more work is needed. Finally, assessment is needed of whether occupational therapy interventions can help to alleviate FOG.

Neural circuits involved in postural control

The neural structures involved in gait are to a certain extent also involved in postural control.[275] Automatic postural responses to a balance perturbation are presumably evoked from the pmRF. The basal ganglia and cortex become involved in the later

phases of the postural response, and are essential when stepping or grasping is required to maintain balance.[176] The scaling of both the automatic postural responses and balance correcting steps is regulated by the basal ganglia and cortex (particularly the supplementary motor area). In addition, spinal interneurons can shape the automatic postural response on the basis of afferent sensory input and descending input from the corticospinal tract.[290,316] Importantly, a recent fMRI-study showed that the neural structures involved in postural control are spatially distinct but contiguous to those in gait.[123] This notion is in line with chapter 9 where we reported distinct differences between PD-patients with postural instability and those with FOG.

Balance impairments in Parkinson's disease

As described in chapter 9, small amplitudes of automatic postural responses and small balance correcting steps contribute to the balance impairments in PD. The precise mechanism underlying the underscaling of balance correcting responses in these patients is not completely clear and need to be unraveled by future studies. The underscaling could result from hypoactivity in the supplementary motor cortex.[90,175] This explanation, however, raises the question why dopaminergic medication has only a small[23,62] or no effect [85,191,192] on balance responses, whereas it does improve supplementary motor cortex activity.[247]

The minor effects of dopaminergic medication on balance impairments could indicate that lesions in non-dopaminergic pathways contribute to postural instability in PD. Deficiencies in cholinergic pathways might be considered, as degeneration of cholinergic neurons is associated with falls,[179] and treatment with the acetylcholinesterase inhibitor donepezil reduced the number of falls in PD-patients in one study.[71] Moreover, the potential contribution of PPN-cholinergic neurons to postural instability in PD has been suggested by a recent PET-study.[244] Although deficits in non-dopaminergic pathways seem to be of importance with regard to balance impairments in PD, the marginal effects of dopaminergic medication do not necessarily preclude a role for dopamine deficiency in the underlying pathophysiology, because the threshold for therapeutic relief may simply be higher than for other symptoms.[142] In the coming years, randomized clinical trials on the effect of cholinesterase inhibitors are expected to be published, which will hopefully contribute to our understanding of cholinergic deficits contributing to postural instability in PD. In addition, a recent study introduced motor imagery as a new model to study the neural of control of balance, which method is expected to further identify neural substrates contributing to balance impairments in PD. [123]

Motor control by the reticular formation

Above, the presumed contribution of the reticular formation to gait and postural control has been described. There is increasing evidence that the reticular formation

is also able to exert control over movements of distal musculature.[12,45,204] In our StartReact study in patients with HSP described in chapter 5, I provided further evidence for the existence of reticulospinal control over coordinated movements of the hand and foot. Reticulospinal motor control to distal movements was first suggested by the work of Lawrence and Kuypers, who made corticospinal and rubrospinal lesions in primates, whereas the reticulospinal pathway remained intact.[202] The animals lost their ability to pick up food, but surprisingly, they were capable of climbing around their cages, which required the ability to strongly grip the cage bars. This observation suggested that there is reticulospinal motor control over distal movements, but that the extent to which the reticulospinal tract is involved depends on the type of movement. Likely, the reticular formation is involved in grasping, but not in all tasks that require individuated finger movements.[166]

Reticulospinal innervation pattern

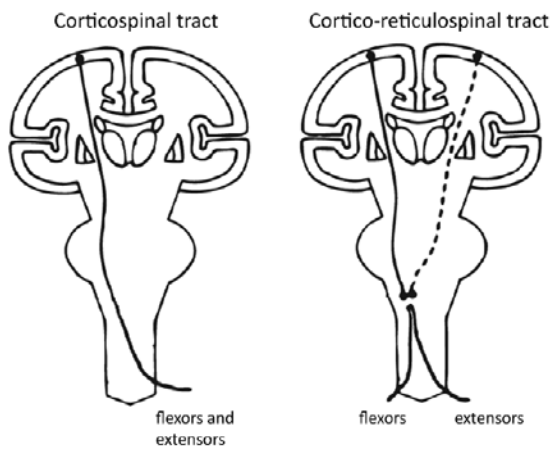
The notion that the reticulospinal tract does not produce individuated fingers movements, distinguishes it from the corticospinal tract, which generates precise movements. This difference between the two tracts can be explained by their output to spinal motoneurons.[12] The reticulospinal axons branch more extensively within the spinal cord compared to corticospinal axons. For example, the same reticulospinal fiber may make contact at both cervical and lumbar level, whereas corticospinal neurons only innervate one segment.[226,227,283] Hence, the activation of reticulospinal fibers does not result in fine fractionated movements, but rather generates a movement that involves multiple muscles around different joints.[12]

Compensatory role of the reticular formation

As the reticular formation is involved in motor preparation (see chapter 5) and the reticulospinal tract innervates both proximal and distal musculature, it could play a compensatory role in the recovery after corticospinal lesions.[12,36] Compensation by the reticulospinal tract and cortico-reticular pathway would primarily require strengthening of the output, not the growth of new neural connections. In chapter 5, I have suggested that in the case of HSP there may be some degree of neuro-plasticity through the reticulospinal system, thereby bypassing the dysfunctional corticospinal tract. In addition, recent experiments showed that the reticulospinal tract might also be responsible for some functional recovery observed after acute corticospinal lesions, such as after stroke.[12] After stroke, a predominant recovery of upper extremity flexor function and lower extremity extensor function is observed. The cortico-reticular and reticulospinal innervation pattern could form an explanation for the flexor bias in the affected upper extremity after stroke, in addition to the extensor bias in the affected lower extremity. Cortico-reticular projections are predominantly ipsilaterally organized, both in healthy subjects[388] and in patients with

stroke.[320,321,384] Furthermore, the reticulospinal neurons innervate the arm flexors and leg extensors ipsilaterally and the arm extensors and leg flexors contralaterally, [81,155] in contrast to corticospinal neurons who innervate both flexors and extensors contralaterally (see figure 2).

Figure 2 Innervation of the upper extremity by the corticospinal and cortico-reticulospinal tract. For the lower extremity the reticulospinal innervation pattern is reversed; so flexors are innervated contralaterally and extensors ispilaterally.



In the case of a supratentorial stroke, there is diminished or absent corticospinal output to contralateral flexors and extensors. Contralesional strengthening of cortico-reticular output might result in larger output to contralesional flexors of the upper extremity, not to contralesional extensors of the upper extremity. Ispilesional strengthening of cortico-reticular output would result in a larger output to contralesional extensors, but the ipsilesional cortico-reticular pathways are often affected after stroke. For the lower extremity the reticulospinal innervation pattern is reversed compared to the upper extremity, innervating flexors contralaterally and extensors ipsilaterally, which might explain the extensor bias seen in the lower extremity after stroke.

Facilitation of reticulospinal compensation

An exciting question is whether compensation by the reticulospinal tract can be stimulated. In chapter 4, I reported that subcortical structures, in particular the reticular formation, can be facilitated by transcranial direct current stimulation (tDCS). The application of tDCS may increase the activation of reticulospinal motoneurons or result in a stronger reticulospinal output, both of which could be beneficial for motor

recovery and successful rehabilitation.[36] The mechanisms underlying the facilitation of subcortical motoneurons are not clear yet. The applied current could have a direct effect on the reticular formation, or facilitation could be the result of an increased cortico-reticular drive to the reticular formation. As it is unknown whether facilitation of the reticular formation by tDCS depends on intact cortico-reticular structures, future studies that make use of diffuse tensor imaging (DTI) could first investigate whether subcortical structures can also be facilitated in people with cortico-reticular lesions. I hypothesize that compensation by the reticular formation does at least partly depend on intact cortico-reticular pathways as patients with HSP, in whom the cortico-reticular pathways are intact, are generally more successful in making voluntary movements compared to stroke patients, in whom the cortico-reticular pathways are affected.

An interesting question that needs to be investigated by future studies is whether reticulospinal compensation contributes to spasticity. Interestingly, patients with HSP exhibit more spasticity when standing compared to lying. The underlying mechanism is unknown, but could be related to enhanced reticulospinal output during stance.

If future studies show that facilitation of the reticulospinal formation facilitates motor recovery after pyramidal lesions, it might be applied in rehabilitation programs. These rehabilitation programs could then make use of recently developed computational models that calculate the effect of lesioned tissue on the current flow during tDCS.[80] Using these computational models, the optimal electrode placement and applied current might be calculated, resulting in individualized tDCS therapy.

The StartReact paradigm

In this thesis, the StartReact paradigm was used to study motor control. In the StartReact paradigm, reaction times are accelerated by a SAS.[54,55,355,356,359] Chapter 5 provides additional evidence for the hypothesis that accelerated reaction times are due to the SAS directly releasing a pre-prepared motor program from subcortical structures. As described in chapter 11, I suggest that cortical and subcortical structures closely interact in motor preparation. Cortically-initiated preparation for motor responses might alter the state of the reticular formation and will result in the representation of a pre-prepared motor response at subcortical level. Future studies need to unravel the exact neural pathways involved in StartReact. I suggest the pmRF as a candidate neural structure in this respect, as it not only constitutes a key structure in the startle reflex circuitry, but has also been demonstrated in studies in monkeys and cats to be involved in motor preparation.[45,315]

Several questions on the involvement of subcortical structures (in particular the reticular formation) in motor preparation remain to be answered. First, it is the question to what extent motor preparation in the reticular formation depends on input from cortico-reticular structures. As I hypothesize that cortical and subcortical structures closely interact during motor preparation, I suggest that intact cortical-reticular connections are very important in sound motor preparation. However, this might partly depend on the type of movement, with subcortical motor preparation of complex movements more heavily depending on input from the cortex compared to simple movements. Secondly, the exact function and benefits of the involvement of the reticular formation in motor preparation is unknown. I hypothesize that the representation of intended movements at reticular level might enhance the postural anticipation of upcoming actions. In addition, I hypothesize that the rapid release of a pre-prepared movement from subcortical structures might have behavioral advantages during situations in which a rapid response to an environmental stimulus is required. Third, the question remains whether subcortical structures are already involved in motor preparation when a movement is performed without previous practice, or in contrast, that an reference copy of the movement becomes available at subcortical level after the movement has been performed. We have recently investigated this hypothesis by evaluating whether StartReact effects are present in unpracticed movements. Our results suggest that the reticular formation is involved in motor preparation of unpracticed movements.

Conclusion

Motor control of gait and balance involves the complex interplay between various neural structures. The reticular formation is one of those. Balance and gait impairments in neurodegenerative diseases are the sum of primary impairments and secondary compensatory mechanisms. Management of gait and balance disorders should try to reduce the primary impairments and to fully enhance compensatory mechanisms. The reticular formation likely contributes to gait deficits in extrapyramidal neuro-degenerative diseases, but is thought to play a compensatory role in patients with pyramidal diseases. Future studies could evaluate whether this compensatory role can be enhanced, for example by tDCS-induced subcortical facilitation.

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Nederlandse samenvatting

Dit hoofdstuk geeft een Nederlandse samenvatting van dit proefschrift, en is vooral gericht op lezers zonder uitgebreide achtergrondkennis. De resultaten worden gedetailleerder bediscussieerd in het hoofdstuk 'Summary and General Discussion'.

Veel neurologische ziekten leiden tot problemen in het aansturen van bewegingen en tot loop- en balansproblemen in het bijzonder. Hierdoor worden mensen minder mobiel en daardoor minder zelfstandig. Daarnaast leiden loop- en balansproblemen vaak tot een val, waardoor mensen blessures kunnen oplopen. Het is daarom niet verrassend dat loop- en balansproblemen tot een verminderde kwaliteit van leven leiden. Helaas zijn de onderliggende mechanismen van loop- en balansproblemen niet geheel duidelijk, en zijn effectieve behandelopties beperkt. Daarom is er meer inzicht nodig in de onderliggende mechanismen. In dit proefschrift wordt lopen en balanshandhaving onderzocht bij drie groepen: (1) gezonde mensen, (2) mensen met hereditaire spastische paraplegie (HSP), en (3) mensen met de ziekte van Parkinson. Studies bij gezonde mensen waren noodzakelijk om meer inzicht te krijgen in de wijze waarop een gezond zenuwstelsel lopen en balanshandhaving aanstuurt. HSP en de ziekte van Parkinson zijn beide neurodegeneratieve aandoeningen. Bij beide ziektebeelden nemen de symptomen in de loop van de tijd toe. Bij HSP en de ziekte van Parkinson zijn echter verschillende onderdelen van het zenuwstelsel aangedaan: het zogenoemde 'pyramidebaansysteem' (HSP) en het 'extrapyramidale systeem' (ziekte van Parkinson). Door mensen met HSP en de ziekte van Parkinson te bestuderen kon worden onderzocht hoe dysfunctie van verschillende onderdelen van het zenuwstelsel bijdraagt aan loop- en balansproblemen. Dit proefschrift richt zich in het bijzonder op de bijdrage van een hersenstamstructuur (*de reticulaire formatie*) aan loop- en balansproblemen. Om de rol van de hersenstam te bestuderen werd in veel studies gebruik gemaakt van de schrikreflex en het 'StartReact' fenomeen.

Deel 1: Gezonde mensen

Schrikreflex

Iedereen schrikt wel eens van een onverwacht luid geluid of van een onverwachte beweging. Het resultaat is een schrikreflex; hierbij worden onbewust spieren aangespannen, allereerst in de nek en daarna in de romp en ledematen. De beenspieren worden vrijwel alleen aangespannen als iemand staat, en niet als iemand zit of ligt. De Engelse term voor een schrikreactie is 'startle reflex' en een luid geluid waarmee een schrikreactie op te wekken is heet een 'startle'. Door middel van de schrikreflex probeert het lichaam zich te beschermen in gevaarlijke situaties. Eén van de doelen hierbij is waarschijnlijk het handhaven van de balans. Omdat dit alleen nodig is als iemand staat, wordt dergelijke reflexactiviteit in de benen slechts gezien

als iemand daadwerkelijk op zijn benen staat. De schrikreflex ontstaat doordat gebieden in de hersenstam geactiveerd worden (*de reticulaire formatie*), die vervolgens spieren aansturen via afdalende zenuwbanen (*de reticulospinale banen*). In hoofdstuk 2 beschrijven we dat spinale interneuronen (zenuwcellen in het ruggenmerg) de reflexactiviteit waarschijnlijk kunnen aanpassen op basis van informatie vanuit receptoren in de benen. Deze receptoren meten hoeveel gewicht iemand met zijn benen draagt, en dus indirect of iemand wel of niet staat.

StartReact fenomeen

Door middel van een 'startle' zijn reactietijden te versnellen. Dit noemt men het 'StartReact' fenomeen. Wanneer een proefpersoon gevraagd wordt om zo snel mogelijk zijn of haar voet op te tillen als er een lampje gaat branden, dan reageert deze na ongeveer 150 ms. Wanneer tegelijkertijd met het lampje een startle gegeven wordt, dan versnelt de reactietijd tot ongeveer 75 ms. Deze versnelling wordt ook gezien bij andere bewegingen zoals starten met lopen, of opstaan uit een stoel. In hoofdstuk 3 onderzoek ik of het ook mogelijk is om met een startle balansreacties te versnellen. Het bleek inderdaad mogelijk om balansreacties op achterwaartse balansverstoringen te versnellen met een startle, maar balansreacties op voorwaartse balansverstoringen bleken niet te versnellen. Dit suggereert dat bij voor- en achterwaartse balansverstoringen verschillende neurale circuits betrokken zijn. Verder suggereren deze resultaten dat er mogelijk een overlap is tussen hersengebieden die betrokken zijn bij schrikreacties en gebieden die zorg dragen voor achterwaartse balansreacties.

Beïnvloeden van hersenstamstructuren

In hoofdstuk 4 wordt onderzocht of de hersenstam, in het bijzonder de reticulaire formatie, beïnvloed kan worden door middel van transcraniële gelijkstroom stimulatie (tDCS). tDCS is een techniek die het mogelijk maakt om de hersenen van buitenaf te stimuleren. Bij tDCS krijgen proefpersonen twee elektroden op het hoofd. Tussen deze elektroden loopt een zwakke gelijkstroom, die de activiteit van hersengebieden enigszins kan doen toe- of afnemen. In hoofdstuk 4 laten we zien dat tDCS zowel balansreacties als het StartReact-fenomeen doet versnellen. Aangezien zowel balansreacties als het StartReact fenomeen waarschijnlijk ontstaan door activatie van de reticulaire formatie, is het aannemelijk dat tDCS zelfs invloed heeft tot op het niveau van de hersenstam.

Deel 2: Hereditaire spastische paraplegie

Box 1 Hereditaire spastische paraplegie (HSP)

Hereditaire spastische paraplegie is een erfelijke ziekte die gepaard gaat met stijfheid (spasticiteit) en zwakte van de beenspieren. Hierdoor ontstaan loopproblemen, die toenemen in de loop van de tijd. Daarnaast hebben mensen met HSP last van balansproblemen. Grofweg zijn er twee groepen HSP te onderscheiden: pure HSP en complexe HSP. Mensen met pure HSP hebben alleen stijfheid en zwakte van de beenspieren. Bij complexe HSP treden ook andere symptomen op zoals dementie en epilepsie. HSP heeft verschillende overervingsvormen: een dominante vorm (generatie op generatie, 50% kans op doorgeven van de ziekte), een recessieve vorm (komt alleen in één generatie voor) en een geslachtsgebonden vorm (treedt alleen op bij mannen). Waarschijnlijk ontstaat HSP doordat transport van stoffen in de zenuwbanen niet optimaal verloopt. Deze transportproblemen treden voornamelijk op aan het uiteinde van de langste zenuwbanen. Daarom hebben patiënten met HSP voornamelijk klachten in de benen en niet in de armen. De ziekteprogressie bij HSP kan niet gestopt worden. Wel kan worden geprobeerd om de symptomen te verlichten. In dit proefschrift werd onderzoek gedaan naar mensen met een pure vorm van HSP en een dominante overervingsvorm.

Mechanisme achter het StartReact fenomeen

Om het mechanisme achter het StartReact effect beter te snappen werd in hoofdstuk 5 het StartReact fenomeen getest bij mensen met HSP. Door het StartReact fenomeen te evalueren bij mensen met HSP konden we onderzoeken of het piramidebaansysteem (*corticospinale banen*) of de reticulospinale banen betrokken zijn bij het StartReact fenomeen. De resultaten laten zien dat het StartReact fenomeen verloopt via de reticulospinale banen. Dit suggereert dat de hersenstam niet alleen betrokken is bij het verwerken van schrikreacties, maar ook bij de voorbereiding van willekeurige bewegingen. Wanneer iemand van plan is om een beweging uit te voeren, wordt het 'programma' voor deze beweging waarschijnlijk vanuit de hersenschors 'aangeboden' aan de hersenstam. Een startle kan dit klaarliggende programma vervolgens vrijmaken, waardoor de beweging sneller dan normaal start.

Balansproblemen bij HSP

In hoofdstuk 6 worden de balansproblemen bij mensen met HSP onderzocht. De resultaten laten zien dat vertraging van spieractivaties in de benen in belangrijke mate bijdraagt aan de balansproblemen bij HSP. Vervolgens werd gezocht naar de oorzaak van de vertraagde balansreacties. Wanneer men uit balans wordt gebracht, sturen opstijgende zenuwbanen in her ruggenmerg (*achterstrengen*) informatie naar de hersenen. De hersenstam zorgt vervolgens voor een evenwichtsreactie door spieren

aan te sturen via afdalende banen (*reticulospinale banen*). Een vertraagde evenwichtsreactie bij mensen met HSP kan ontstaan door een vertraagde geleiding in de opstijgende of afdalende zenuwbanen, of in een combinatie van beide. Om dit uit te zoeken werden balansreacties gecombineerd met een startle. Hierdoor kon het 'programma' voor de evenwichtsreactie direct vanuit de hersenstam worden opgewekt, zonder dat hiervoor informatie vanuit de opstijgende zenuwbanen nodig was. Wanneer de balansverstoring namelijk gecombineerd werd met een luide geluidstoon versnelden de evenwichtsreacties, zowel bij mensen met HSP als bij gezonde controledeelnemers. Echter, de versnelling was groter bij mensen met HSP, waardoor er geen verschil in reactiesnelheid meer was tussen beide groepen. Het lijkt er dus op dat er bij HSP sprake is van een vertraging in de opstijgende zenuwbanen die aan de hersenen doorgeven dat er een balansverstoring is. Doordat de hersenen nét iets later weten dat er een balansverstoring is, zal de uiteindelijke evenwichtsreactie vertraagd zijn.

Compensatie via de reticulaire formatie

Bij mensen met HSP is een deel van de zenuwbanen aangedaan (*corticospinale banen, achterstrengen*). Dit proefschrift laat zien dat de banen vanuit de hersenstam (*reticulospinale banen*) zeer waarschijnlijk niet zijn aangedaan. Mogelijk gebruiken mensen met HSP deze reticulospinale banen in toenemende mate om bewegingen uit te voeren. Recente studies laten zien dat dit mogelijk ook gebeurt bij mensen die een hersenbloeding of -infarct hebben doorgemaakt. Het is de vraag of deze vorm van compensatie bevorderd kan worden, wat tot een beter herstel zou kunnen leiden. In dit proefschrift laten we zien dat de reticulaire formatie bij gezonde mensen te stimuleren is met tDCS. Het is de vraag of dit ook mogelijk is bij mensen met HSP en bij mensen die een hersenbloeding of -infarct hebben doorgemaakt. Als dit zo is, zou tDCS mogelijk toegepast kunnen worden om compensatie via de reticulaire formatie te bevorderen, hopelijk resulterend in een verbetering van het lopen en de balanshandhaving.

Deel 3: Ziekte van Parkinson

Box 2 Ziekte van Parkinson

Bij de ziekte van Parkinson sterven bepaalde hersencellen af. Deze cellen bevinden zich in de substantia nigra en maken de stof dopamine. Een tekort aan dopamine leidt tot de volgende motorische symptomen: trillen (tremor), stijfheid (rigiditeit), vertragen van bewegingen (bradykinesie) en verminderd aanwezig zijn van bewegingen (hypokinesie). Als de ziekte vordert kunnen ook balansproblemen en 'bevriezen' van het lopen ontstaan. De ziekte van Parkinson kan ook leiden tot niet-motorische symptomen zoals een verminderd reukvermogen, stemmingsstoornissen, geheugenstoornissen, moeite met logisch redeneren en slaapstoornissen. Er zijn nog geen behandelingen waarmee de ziekte van Parkinson gestopt kan worden. Wel is het mogelijk om de symptomen te onderdrukken of te compenseren. Dit gebeurt voornamelijk door middel van fysieke training (o.a. fysiotherapie), ergotherapie, medicamenten (medicijnen die lijken op dopamine) en hersenoperaties (diepe hersenstimulatie).

Balansproblemen bij de ziekte van Parkinson

In hoofdstuk 7 wordt beschreven hoe balansproblemen kunnen worden onderzocht met dynamische posturografie. Dynamische posturografie is een methode waarbij mensen uit balans worden gebracht. Vaak gebeurt dit door middel van een bewegend platform. Met verschillende meetapparatuur kunnen de opgewekte balansreacties vervolgens bestudeerd worden.

Bevriezen van lopen bij de ziekte van Parkinson

Bevriezen van lopen komt voor bij een deel van de mensen met de ziekte van Parkinson. Tijdens bevriezen van lopen hebben mensen het gevoel dat hun voeten plotseling vastgeplakt raken aan de grond. Hierdoor lukt het niet om een stap te maken, of maken mensen heel snelle stapjes op de plaats. Bevriezen van lopen treedt voornamelijk op tijdens het starten en tijdens het draaien. Het onderliggende mechanisme is nog onduidelijk. In hoofdstuk 8 wordt de hypothese onderzocht dat bevriezen van lopen ontstaat doordat gebieden in de hersenstam de verschillende componenten van het lopen niet goed coördineren. Deze hersenstamgebieden werden getest door het StartReact fenomeen toe te passen op het starten met lopen. Het bleek dat het versnellende effect van een startle verminderd is bij mensen die last hebben van bevriezen. Naast een verminderd StartReact fenomeen, maakten deze mensen ook kleinere stapjes dan mensen die geen last hadden van bevriezen. Ook was de gewichtsverplaatsing die voorafgaat aan een stap minder bij de bevriezers. Het verminderde StartReact effect bij de bevriezers laat zien dat bepaalde hersenstamgebieden niet goed functioneren. Mogelijk is het 'bewegingsprogramma' voor het lopen bij bevriezers onvoldoende aanwezig op hersenstamniveau, of hebben zij

moeite om dit vrij te maken. Hierdoor verloopt de coördinatie van de verschillende onderdelen van een stap minder goed. Aangezien bevriezers kleinere stappen maken en een kleinere gewichtsverplaatsing voorafgaande aan de stap hebben, wordt de coördinatie van de verschillende onderdelen van een stap juist belangrijker. Mogelijk leidt een onvermogen om de verschillende staponderdelen goed te coördineren tot bevriezen van lopen.

Relatie bevriezen van lopen en balansproblemen

Omdat bevriezen van lopen vaak samen gaat met balansproblemen werd in hoofdstuk 9 onderzocht of dysfunctie van hersenstamgebieden ook bijdraagt aan de balansproblemen bij de ziekte van Parkinson. Dit werd gedaan door te beoordelen of bij mensen met Parkinson achterwaartse balansreacties kunnen worden versneld door middel van een startle. Dit bleek inderdaad mogelijk bij mensen met balansproblemen, maar niet bij de mensen die (ook) last hadden van bevriezen. Op basis hiervan is het niet waarschijnlijk dat balansproblemen bij de ziekte van Parkinson ontstaan door de hersenstamproblemen die leiden tot bevriezen. De balanscorrigerende reacties bleken veel kleiner bij mensen met balansproblemen dan bij de mensen zonder balansproblemen. Doordat zij kleinere balanscorrigerende stappen zetten, moesten mensen met balansproblemen veel meer stappen zetten om niet te vallen. Dit past bij het idee dat vooral te kleine balansreacties bijdragen aan de balansproblemen bij de ziekte van Parkinson.

Behandelprotocol voor bevriezen van lopen

In dit proefschrift is onder andere onderzoek gedaan naar de onderliggende mechanismen van bevriezen van lopen. Hopelijk draagt het resultaat hiervan in de toekomst bij aan verbeterde behandelopties. Momenteel kan bevriezen van lopen niet worden genezen. Wel kan worden geprobeerd om de symptomen te verminderen. De behandeling van bevriezen van lopen wordt in het algemeen als erg lastig ervaren. Er zijn weliswaar veel behandelopties, zoals verschillende soorten medicijnen, hersenoperaties, fysiotherapie en ergotherapie, maar het wetenschappelijke bewijs voor deze behandelopties is vaak nog onvoldoende en een duidelijk behandelprotocol ontbreekt. In hoofdstuk 10 werd daarom met een internationale groep experts een behandelprotocol voor bevriezen van lopen bij de ziekte van Parkinson opgesteld. Dit protocol is tot stand gekomen door het wetenschappelijk bewijs van iedere behandeloptie te beoordelen. Daarnaast is ook de persoonlijke expertise van de auteurs meegenomen. Het resultaat is een artikel met tips voor behandelaars en een stroomschema met behandelopties dat gebruikt kan worden in de dagelijkse klinische praktijk.

Deel 4: Overzicht

In hoofdstuk 11 wordt beschreven wat schrikreacties en het StartReact fenomeen ons vertellen over de wijze waarop de hersenen balansreacties en het lopen aansturen. Allereerst hebben balansreacties en schrikreacties overeenkomstige kenmerken, en ontstaan mogelijk vanuit dezelfde hersenstamstructuren. Daarnaast tonen studies die gebruik maken van het StartReact fenomeen aan dat de hersenschors (*cortex*) en hersengebieden onder de cortex (*subcorticale structuren*, onder andere de hersenstam) zeer nauw samenwerken bij het aansturen van balansreacties en het lopen.

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List of publications

1. **Nonnekes J**, Talelli P, de Niet M, Reynolds RF, Weerdesteyn V, Day BL. Deficits underlying impaired visually triggered step adjustments in mildly affected stroke patients. Neurorehabilitation and neural repair 2010;24:393-400.
2. Snijders AH, **Nonnekes J**, Bloem BR. Recent advances in the assessment and treatment of falls in Parkinson's disease. F1000 medicine reports 2010;2:76.
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19. Dibilio V, Stummer C, Drenthen L, Bloem BR, **Nonnekes J**, Weerdesteyn V. Secondary task performance during challenging walking tasks and freezing episodes in Parkinson's disease. *Submitted*.
20. Zach H, Janssen AM, Snijders AH, Delval A, Ferraye MU, Auff E, Weerdesteyn V, Bloem BR, **Nonnekes J**. Indentifying freezing of gait in Parkinson's disease using waist-mounted accelerometry. *Submitted*.

Curriculum vitae

Jorik Nonnekes was born in Zaltbommel on November 21, 1985. After graduating from secondary school (Cambium College, Zaltbommel), he started to study medicine at the Radboud University Nijmegen. Jorik performed a scientific elective at the Institute of Neurology, Queen Square in London in 2008. Under supervision of prof. Brian Day he studied visually triggered step adjustments in chronic stroke patients. Jorik graduated in 2010, and obtained both his bachelor and master certificate cum laude. After graduating, he worked at the emergency department of the Jeroen Bosch Hospital. At the same time, he wrote together with dr. Vivian Weerdesteyn, prof. Sander Geurts and prof. Bas Bloem a proposal for research on gait and balance impairments in patients with Parkinson's disease. With this proposal, he participated in a PhD-competition for talented master students at the Radboud University Medical Centre. Via this competition he obtained a personal PhD-grant in 2011, and was able to start with his PhD-project. From 2013 onwards, Jorik combined his research with working as a resident at the Sint Maartenskliniek, and as a resident at the department of clinical neurophysiology of the Radboud University Medical Centre. Moreover, he participated in the Radboud Da Vinci Challenge, a program that offers excellent PhD students and post-docs the opportunity to experience broad personal development. Currently, Jorik works as a resident in rehabilitation medicine. In addition, he continues his research on gait and balance impairments by working one day a week as a post-doc in the group of prof. Richard van Wezel. Jorik lives together with Femke Nieuwenhuis.

Curriculum vitae

Jorik Nonnekes is geboren op 21 november 1985 in Zaltbommel. In 2004 behaalde hij zijn VWO diploma aan het Cambium College in Zaltbommel, waarna hij begon met de studie geneeskunde aan de Radboud Universiteit Nijmegen. Begin 2008 verrichte Jorik zijn wetenschappelijk stage aan het Insitute of Neurology, Queen Square in Londen. Onder supervisie van prof. Brian Day bestudeerde hij visueel-getriggerde stapaanpassingen bij chronische CVA-patiënten. Jorik ronde zijn opleiding af in 2010; zowel zijn bachelor- als masterdiploma behaalde hij cum laude. Hierna werkte hij een half jaar als poortarts op de spoedeisende hulp van het Jeroen Bosch Ziekenhuis. Ondertussen schreef hij samen met dr. Vivian Weerdesteyn, prof. Sander Geurts en prof. Bas Bloem een onderzoeksvoorstel naar loop- en balansproblemen bij de ziekte van Parkinson. Met dit onderzoeksvoorstel deed hij mee aan een PhD-competitie voor talentvolle masterstudenten binnen het Radboud UMC. In 2011 verkreeg hij via deze competitie een persoonlijke PhD-subsidie, en startte hij met zijn promotietraject. Vanaf 2013 combineerde Jorik zijn onderzoek met het werk als arts-assistent in de Sint Maartenskliniek en daarna als arts-assistent op de afdeling klinische neurofysiologie van het Radboud UMC. Daarnaast nam hij deel aan de Radboud Da Vinci Challenge, een programma voor excellente promovendi en post-docs gericht op een brede persoonlijke ontwikkeling. Momenteel werkt Jorik als revalidatiearts in opleiding binnen het circuit Nijmegen-Den Bosch. Zijn onderzoek naar loop- en balansproblemen zet hij voort door één dag per week als post-doc te werken in de groep van prof. Richard van Wezel. Jorik woont samen met Femke Nieuwenhuis.

Dissertations of the Disorders of Movement Research Group, Nijmegen

Parkinson Center Nijmegen (ParC)

1. Jasper E. Visser. The basal ganglia and postural control. Radboud University Nijmegen, 17 June 2008
2. Maaike Bakker. Supraspinal control of walking: lessons from motor imagery. Radboud University Nijmegen, 27 May 2009
3. W. Farid Abdo. Parkinsonism: possible solutions to a diagnostic challenge. Radboud University Nijmegen, 7 October 2009
4. Samyra H.J. Keus. Physiotherapy in Parkinson's disease: towards evidence-based practice. Leiden University, 29 April 2010
5. Lars B. Oude Nijhuis. Modulation of human balance reactions. Radboud University Nijmegen, 29 November 2010
6. Maarten J. Nijkrake. Improving the quality of allied health care in Parkinson's disease through community-based networks: the ParkinsonNet health care concept. Radboud University Nijmegen, 29 November 2010
7. Rick C.G. Helmich. Cerebral reorganization in Parkinson's disease. Radboud University Nijmegen, 24 May 2011
8. Charlotte A. Haaxma. New perspectives on preclinical and early stage Parkinson's disease. Radboud University Nijmegen, 6 December 2011
9. Johanna G. Kalf. Drooling and dysphagia in Parkinson's disease. Radboud University Nijmegen, 22 December 2011
10. Anke H. Snijders. Tackling freezing of gait in Parkinson's disease. Radboud University Nijmegen, 4 June 2012
11. Bart F.L. van Nuenen. Cerebral reorganization in premotor parkinsonism. Radboud University Nijmegen, 22 November 2012
12. Wandana Nanhoe-Mahabier. Freezing and falling in Parkinson's disease: from the laboratory to the clinic. Radboud University Nijmegen, 13 February 2012
13. Marlies van Nimwegen. Promotion of physical activity in Parkinson's disease, the challenge to change behavior. Radboud University Nijmegen, 6 March 2013
14. Arlène D. Speelman. Promotion of physical activity in Parkinson's disease, feasibility and effectiveness. Radboud University Nijmegen, 6 March 2013
15. Tjitske Boonstra. The Contribution of each leg to bipedal balance control. University Twente, 6 June 2013
16. Marjolein A. Van der Marck. The many faces of Parkinson's disease: towards a multifaceted approach? Radboud University Nijmegen, 10 January 2014
17. Katrijn Smulders. Cognitive control of gait and balance in patients with chronic stroke and Parkinson's disease. Radboud University Nijmegen, 21 May 2014
18. Marjolein B. Aerts. Improving diagnostic accuracy in parkinsonism. Radboud University Nijmegen, 27 June 2014
19. Maartje Louter. Sleep in Parkinson's disease. A focus on nocturnal movements. Radboud University Nijmegen, 13 February 2015
20. Frederick J.A. Meijer. Clinical Application of Brain MRI in Parkinsonism: From Basic to Advanced Imaging. Radboud University Nijmegen, 23 June 2015
21. Jorik Nonnekes. Balance and gait in neurodegenerative disease: what startle tells us about motor control. Radboud Unversity Nijmegen, 2 september 2015

Non-parkinsonian disorders of movement

1. Sascha Vermeer. Clinical and genetic characterization of autosomal recessive cerebellar ataxias. Radboud University Nijmegen, 5 April 2012
2. Susanne T. de Bot. Hereditary spastic paraplegias in the Netherlands. Radboud University Nijmegen, 20 December 2013
3. Catherine C.S. Delnooz. Unravelling primary focal dystonia. A treatment update and new pathophysiological insights. Radboud University Nijmegen, 7 January 2014

Vascular disorders of movement – The Radboud Stroke Centre

1. Liselore Snaphaan. Epidemiology of post-stroke behavioural consequences. Radboud University Nijmegen, 12 March 2010
2. Karlijn F. de Laat. Motor performance in individuals with cerebral small vessel disease: an MRI study. Radboud University Nijmegen, 29 November 2011
3. Anouk G.W. van Norden. Cognitive function in elderly individuals with cerebral small vessel disease. An MRI study. Radboud University Nijmegen, 30 November 2011
4. Rob Gons. Vascular risk factors in cerebral small vessel disease. A diffusion tensor imaging study. Radboud University Nijmegen, 10 December 2012
5. Loes C.A. Rutten-Jacobs. Long-term prognosis after stroke in young adults. Radboud University Nijmegen, 14 April 2014
6. Noortje Maaijwee. Long-term neuropsychological and social consequences after stroke in young adults. Radboud University Nijmegen, 12 june 2015

Neuromuscular disorders of movement

1. Mireille van Beekvelt. Quantitative near infrared spectroscopy (NIRS) in humanskeletal muscle. Radboud University Nijmegen, 24 April 2002
2. Johan Hiel. Ataxia telangiectasia and Nijmegen Breakage syndrome, neurological, immunological and genetic aspects. Radboud University Nijmegen, 23 April 2004
3. Gerald JD Hengstman. Myositis specific autoantibodies, specificity and clinical applications. Radboud University Nijmegen, 21 September 2005
4. M. Schillings. Fatigue in neuromuscular disorders and chronic fatigue syndrome, a neurophysiological approach. Radboud University Nijmegen, 23 November 2005
5. Bert de Swart. Speech therapy in patients with neuromuscular disorders and Parkinson's disease. Diagnosis and treatment of dysarthria and dysphagia. Radboud University Nijmegen, 24 March 2006
6. J. Kalkman. From prevalence to predictors of fatigue in neuromuscular disorders. The building of a model. Radboud University Nijmegen, 31 October 2006
7. Nens van Alfen. Neuralgic amyotrophy. Radboud University Nijmegen, 1 November 2006
8. Gea Drost. High-density surface EMG, pathophysiological insights and clinical applications. Radboud University Nijmegen, 9 March 2007
9. Maria Helena van der Linden. Perturbations of gait and balance: a new experimental setup applied to patients with CMT type 1a. Radboud University Nijmegen, 6 October 2009
10. Jeroen Trip. Redefining the non-dystrophic myotonic syndromes. Radboud University Nijmegen, 22 January 2010
11. Corinne Horlings. A weak balance, balance and falls in patients with Neuromuscular disorders. Radboud University Nijmegen, 1 April 2010
12. E. Cup. Occupational therapy, physical therapy and speech therapy for persons with Neuromuscular diseases, an evidence based orientation. Radboud University Nijmegen, 5 July 2011
13. Alide Tieleman. Myotonic dystrophy type 2, a newly diagnosed disease in the Netherlands. Radboud University Nijmegen, 15 July 2011
14. Nicol Voermans. Neuromuscular features of Ehlers-Danlos syndrome and Marfan syndrome. Radboud University Nijmegen, 2 September 2011
15. Allan Pieterse. Referral and indication for occupational therapy, physical therapy and speech-language therapy for persons with neuromuscular disorders. Radboud University Nijmegen, 13 February 2012
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